EVALUATION OF DIFFERENT COATING MATERIALS FOR ENCAPSULATION OF PHENOLIC COMPOUNDS EXTRACTED FROM ONION (*Allium Cepa*) SKIN

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EVALUATION OF DIFFERENT COATING MATERIALS FOR ENCAPSULATION OF PHENOLIC COMPOUNDS EXTRACTED FROM ONION (*Allium Cepa*) SKIN

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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

EVALUATION OF DIFFERENT COATING MATERIALS FOR ENCAPSULATION OF PHENOLIC COMPOUNDS EXTRACTED FROM ONION (*Allium Cepa*) SKIN

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Encapsulation provides a protective barrier to some vulnerable food ingredients such as phenolic and antioxidant compounds against some external factors. Moreover, by the help of encapsulation the unwanted odor or taste of core the materials are masked.

The main objective of the study was to evaluate the effects of different type of coating materials on encapsulation of phenolic compounds extracted from onion skin. Coating materials were maltodextrin, gum arabic, casein and whey protein concentrate (WPC). As a coating material, maltodextrin was either used alone or it was combined with the other coating materials at ratios of 8:2 and 6:4.

The emulsions were prepared with core to coating ratios of 1:10 and 1:20. Freeze dried capsules were evaluated in terms of total phenolic compounds, antioxidant activity, encapsulation efficiency, particle size distribution, morphology and heat stability.

Total phenolic content and antioxidant activity values were higher in microcapsules with core to coating ratio of 1:10 than microcapsules with core to coating ratio of 1:20. Capsules with core to coating ratio of 1:20 had higher encapsulation efficiency values than capsules with core to coating ratio of 1:10. Combining maltodextrin with casein was effective for keeping the phenolic compounds inside the capsule with its higher efficiency values (84.39-89.15%).

Particle size of the capsules with core to coating ratio of 1:10 were higher in terms of D32 than the ones with core to coating ratio of 1:20. Optical images of the microcapsules confirmed the particle size distribution results. Usage of casein coating were more efficient than whey protein in terms of increasing heat stability of capsules.

Key words: Encapsulation, onion skin, phenolic compounds, encapsulation efficiency, antioxidant activity

ÖZ

SOĞAN KABUĞUNDAN (*Allium Cepa*) ELDE EDİLEN FENOLİK MADDELERİN FARKLI KAPLAMA MALZEMELERİ İLE ENKAPSULASYONUNUN DEĞERLENDİRİLMESİ

Akdeniz, Büşra

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Enkapsülasyon, fenolik ve antioksidan maddeler gibi bazı hassas gıda bileşenlerini dış etmenlere karşı koruyucu bir bariyer sağlar. Ayrıca, enkapsülasyon yardımı ile çekirdek maddelerin istenmeyen koku ve tatları maskelenir.

Bu çalışmanın asıl amacı, soğan kabuğundan elde edilen fenolik maddelerin farklı çeşitteki kaplama malzemeleri ile kaplanmasının değerlendirilmesidir. Kaplama malzemeleri maltodekstrin, arap zamkı, kazein ve peynir altı suyu protein konsantresidir. Kaplama maddesi olarak maltodekstrin ya tek başına

ya da diğer kaplama malzemeleri ile 8:2 ve 6:4 oranları ile karıştırılarak kullanılmıştır.

Emülsiyonlar çekirdek: kaplama malzemesi oranları 1:10 ve 1:20 olacak şekilde hazırlanmıştır. Dondurmalı kurutucuda kurutulan kapsüller, toplam fenolik madde, antioksidan aktivite, enkapsülasyon verimi, parçacık boyutu analizi, morfoloji ve ısı dayanıklılığı açısından değerlendirilmiştir.

Çekirdek: kaplama maddesi oranı 1:10 olan mikrokapsüllerde toplam fenolik madde miktarı ve antioksidan aktivitesi çekirdek:kaplama maddesi oranı 1:20 olan mikrokapsüllere göre daha yüksektir. Çekirdek:kaplama maddesi oranı 1:20 olan kapsüllerin enkapsülasyon verimi çekirdek:kaplama maddesi oranı 1:10 olanlara göre daha yüksektir. Maltodekstrinin kazein ile kombinasyonu yüksek verim değerleri ile fenolik maddeleri kapsül içinde tutabilme açısından etkilidir (84.39-89.15%).

Çekirdek:kaplama malzeme oranı 1:10 olan kapsüllerin D32 değerleri çekirdek:kaplama malzemesi 1:20 olanlara göre daha yüksektir. Optik görüntüler parçacık boyutu dağılımı sonuçlarını doğrulamaktadır. Kaplama maddesinde kazein kullnımı kapsüllerin ısıl dayanıklılığının arttırılması açısından peynir altı suyu proteinine göre daha etkilidir.

Anahtar kelimeler: Enkapsülasyon, soğan kabuğu, fenolik maddeler, enkapsülasyon verimi, antioksidan aktivite

To my beloved family…

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INTRODUCTION

1.1 Phenolic compounds

Plant metabolism is divided into two groups: primary and secondary pathways. Primary pathways deal with the management of basic compounds such as proteins, carbohydrates, lipids and nucleic acids. They act in many reactions like biosynthesis or glycolysis in all cells and they are essential for cell maintenance (Rosa, Alvarez-Parilla, & Gonzalez-Aguilar, 2010). On the other hand, secondary pathways participate in specialized cells and generate various unique compounds like terpenoids, phenolics and alkaloids (Rosa, Alvarez-Parilla, & Gonzalez-Aguilar, 2010). These compounds generally have aromatic ring carrying one or more hydroxyl group. (Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999).

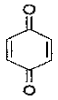
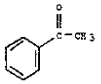
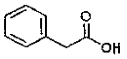
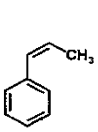
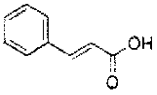
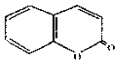
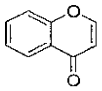
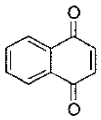
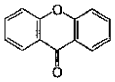
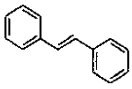
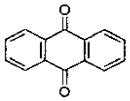
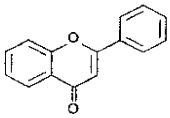
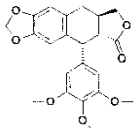
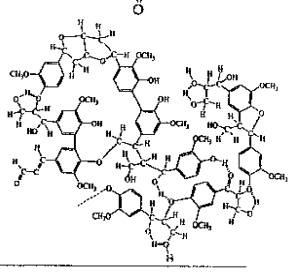
Phenolic compounds play significant role in physiology and cellular metabolism in plants. They have many functions in plants, such as sensory properties (astringency, taste, color, and aroma), pollination, structure, resistance to undesirables (pests and predators), UV light and oxidative damage protectant (Fraga, 2010) Phenolic compounds can be divided into three classes according to their distribution in nature. The shortly distributed ones are simple phenols , aldehydes derived from benzoic acids,

hydroquinone, pyrocatechol, resorcinol, widely distributed ones are flavonoids, phenolic acids and coumarins and polymer ones are lignin and tannin (Vermerris & Nicholson, 2008).

Phenolic compounds have some health promoting and protective effects against degenarative diseases (cardiovascular disease, diabetes, cancer, high-cholesterol levels) (Fraga, 2010). They have stable and powerful antioxidant properties. In other words, they can defend tissues against free radical damage caused by oxidation (Balasundram, Sundram, & Samman, 2006). In addition, phenolic compounds exhibit various physiological properties like anti-microbial, anti-inflammatory, anticarcinogenic, antiallergenic, antimutagenic and anti-thrombotic effects. They can protect DNA from oxidative damage by scavenging Reactive Oxygen Species (Balasundram et al., 2006; Robards et al., 1999). Flavonoids, a subclass of phenolic compounds, have ability to inhibit spore germination of plant pathogens and some researches showed that they show anti-viral effect (Cushnie & Lamb, 2005).

Phenolic substances can be classified into different groups with regard to the number of phenol rings and the structural elements binding these rings (Vermerris & Nicholson, 2008). In Table 1.1, there are some examples for the classification.

Table 1.1 Main classes of phenolic compounds (Fraga, 2010)

Class	Basic skeleton	Basic structure
Simple phenols	C_6	
Benzoquinones	C_6	
Phenolic acids	C_6-C_1	
Acetophenones	C_6-C_2	
Phenylacetic acids	C_6-C_2	
Hydroxycinnamic acids	C_6-C_3	 
Phenylpropenes	C_6-C_3	
Coumarins, isocoumarins	C_6-C_3	
Chromones	C_6-C_3	
Naphthoquinones	C_6-C_4	
Xanthones	C_6-C_1-C_6	
Stilbenes	C_6-C_2-C_6	
Anthraquinones	C_6-C_2-C_6	
Flavonoids	C_6-C_3-C_6	
Lignans and neolignans	$(C_6$-$C_3)_2$	
Lignins	$(C_6$-$C_3)_n$	

1.1.1 Phenolic acids

Phenolic acids are divided into two groups: benzoic acid derivatives which have seven carbon atoms (C6-C1) and cinnamic acid derivatives containing nine carbon atoms (C6-C3). These compounds have carboxylic acid group, a benzene ring and one or more methoxyl and/or hydroxyl groups (Vermerris & Nicholson, 2008). Phenolic acids are generally in bound form conjugating with organic acids and sugar molecules. The antioxidant activity of phenolic acids increases if more hydroxyl groups are attached (Robards et al., 1999).

P-coumaric, caffeic, ferulic, and sinapic acids are the most common group of cinnamic acids. They can be found in both free and esterified form in all parts of the fruit, but the outer region of the ripe fruit has the highest concentration (Mérillon & Ramawat, 2013). The most important member of this group in food material is chlorogenic acid. It is the important substrate for enzymatic browning mostly in pears and apples (Mérillon & Ramawat, 2013). Ferulic acid is the major phenolic acid which is in cell wall structure in trans conformation. It is found mainly in cereals (Fraga, 2010).

In hydroxybenzoic acid group, there are salicylic acid, p-hydroxybenzoic, protocatechuic, gentisic acid, gallic, syringic acid and vanillic acids (Balasundram et al., 2006). Though in some fruits they are in free acid form (gallic acid in persimmons), they are present as conjugate form in general. Gallic acid can also be in conjugated forms named as ellagic acid, gallagic acid and tergallic acid. This partially limited forms are called dimer, trimer and tetramer (Natella, Nardini, Felice, & Scaccini, 1999). Hydrobenzoic acid glycosides are characteristic of some herbs and spices. Despite cinnamic acid derivatives can be found abundant in edible plant sources, the hydrobenzoic acids content is low (Fraga, 2010).

1.1.2 Lignans and stilbenes

Lignans are the type of phenylpropanoid found in plants. Phenylpropanoids are the phenylalanine derived secondary metabolites (Fraga, 2010). They are classified into subgroups (furofuran, furan, aryltetralin, arylnaphthalene, dibenzylbutyrolactone, dibenzylbutyrolactol and dibenzocyclooctadiene) according to oxygen incorporation into skeleton (Rosa, Alvarez-Parilla, & Gonzalez-Aguilar, 2010). Many plant-based foods like seeds, legumes, grains, fruit and vegetables contain lignan precursor. Lignan precursors are metabolized by bacterias in human intestine and used as dietary fiber. Flaxseeds are the richest dietary source of them (Mérillon & Ramawat, 2013).

The stilbene family are phytoalexins (C_6-C_2-C_6 structure) produced by plants in response to injury, stress or disease condition. The main source of stilbenes is resveratrol which is mostly found in wine products. It can be in the form of *cis* and *trans* isomers (Fraga, 2010). Stilbenes are mostly found in vascular type of plants. They show defense mechanism with its fungicidal and antimicrobial effect. Some of them can inhibit spore germination, whereas others have toxic effect to insects and parasitic worms (Rosa, Alvarez-Parilla, & Gonzalez-Aguilar, 2010).

1.1.3 Flavonoids

Flavonoids are the largest group of the phenolic compunds in plants. As consisting of fifteen carbon atoms with C_6-C_3-C_6 skeleton, they are low in molecular weight. They seperate into subgroup of six according to type of

heterocycle (Figure 1.1) which are flavones, flavanols (cathechins & proanthocyanidins), flavanones, flavonols, isoflavonoids (isoflavones), anthocyanidins (Balasundram et al., 2006).

In addition, they are also found in methylated and sulfated derivatives, conjugated with monosaccharides and disaccharides and building up complexes with lipids, oligosaccharides, amines, carboxylic acids and organic acids (Spencer & Crozier, 2012).

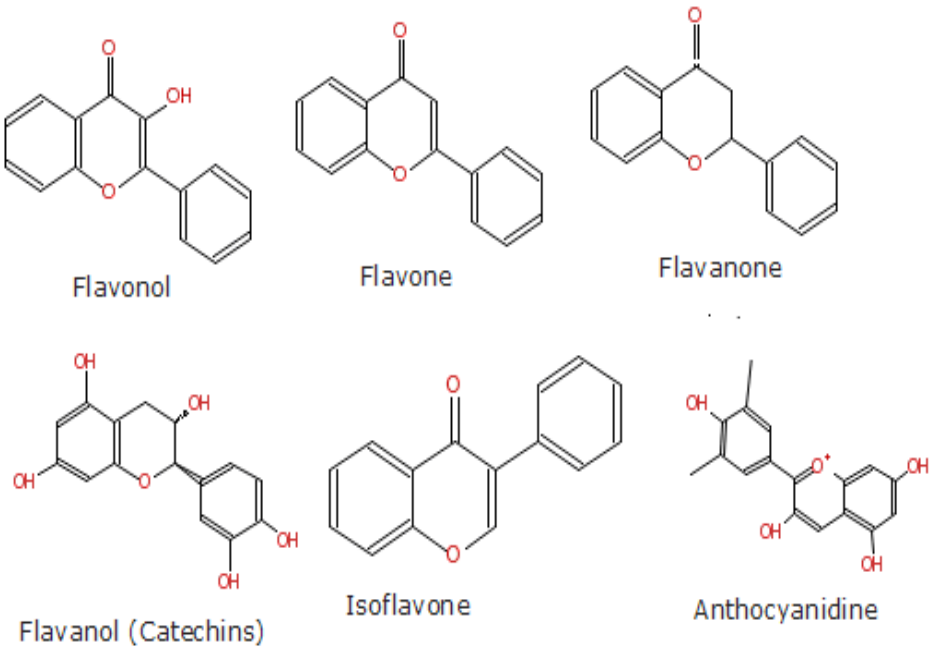


Figure 1.1 Main classes of flavonoids chemical structure (Spencer & Crozier, 2012)

While some classes of flavonoid members are colorless (flavanones), some of them have color pigments (anthocyanins). Anthocyanins are water soluble compounds that are responsible for the purple, blue, and red colors of the plant tissues. The color of the anthocyanidins changes with pH value. At low pH, they are usually red, colorless with sulfur compound and blue when pH increases. They can form complex with metal ions and flavones (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). Anthocyanins are highly susceptible to degredation against light, pH, temperature, ascorbic acid, enzymes, sulfites and oxidation. For this reason, many studies have been conducted to increase the anthocyanin stability. The degradation can be prevented by esterification and glycosylation with organic acids (for example; citric and malic acids) and phenolic acids. Moreover, stabilization of anthocyanins is accomplished by encapsulation and the formation of complexes with other flavonoids (Cavalcanti, Santos, & Meireles, 2011).

Flavonols are represented mainly by kaempferol, myricetin, quercetin and methylated derivative isorhamnetin (Fraga, 2010). Especially quercetin is the most widespread type. These colorless compounds are found many fruits and vegetables like apples, berries, onion, but onion has the highest content of quercetin. Flavonols are concentrated mostly in the form of glycosylated conjugates (such as quercetin-3-glucoside, quercetin-3,4′diglucoside) in plant tissues (Spencer & Crozier, 2012).

Flavones have similar structure with flavonols. The only difference is the absence of hydroxylation at third carbon. If they are in high concentrations or are complexed with metal ions, they help to colorize the plant tissue (Peterson & Dwyer, 1998). Common flavones are existed in glycosylated form in plants (commonly in herbs, vegetables and grains) which are

apigenin and luteolin. Polymeyhoxylated flavones such as sinensetin, nobiletin and tangeretin are found in citrus species and participate in taste development (Fraga, 2010).

Isoflavones are known with their estrogenic activity. In other words, they are in the class of phytoestrogens. Though they are not steroids, they have hydroxyl groups in positions 7 and 4' resembling with the hydroxyls in the estradiol molecule. By this property it shows pseudo-hormonal properties which have ability to bind estrogen receptor (Manach et al., 2004). The leguminous plants contain isoflavones particularly. Soy and its products are the main source. The most common isoflavonoids are genistein and daidzein. The other isoflavones are formononetin and biochanin A (Rosa, Alvarez-Parilla, & Gonzalez-Aguilar, 2010). Since isoflavones are in the form of glycosides in plants, they are highly polar molecules (Setchell & Cassidy, 1999).

Flavanones are represented by an oxygen atom and saturated three carbon series in C4 position (Rosa, Alvarez-Parilla, & Gonzalez-Aguilar, 2010). Their structure is highly reactive that can undergo glycosylation, hydroxylation and O-methylation reactions. Flavanones are mostly found in citrus type of fruits in glycosidic form (Fraga, 2010). In foods, the main flavanones are naringenin, eridictyol and hesperetin. Hesperidin can be found in orange, cumin and peppermint, naringenin is found in grape and lemons contain eridictiol compound. In addition, they can be found in tomatoes and certain aromatic plants like mint (Manach et al., 2004; Peterson & Dwyer, 1998).

The most complex class of flavonoids are flavanols (flavan-3-ols), since flavanols range from simple monomers form (catechins) to polymeric form (proanthocyanidins). There are two chiral centers at the second and third carbon of the monomeric flavanols, these centers produce isomers named as (+) catechins, (-) epicatechins and (-) epiafzelechin. The polymeric proanthocynaidins have extra chiral center at C4 unit (Spencer & Crozier, 2012). In contrast to other classes of flavonoid molecules, they are in aglycone form i.e., they are not in glycosylated form (Rosa, Alvarez-Parilla, & Gonzalez-Aguilar, 2010). The concentration of flavanol molecules are higher in immature fruit. Catechins are more abundant in the outer tissues. Catechin and epicatechin are the most common flavanols in fruits. In addition, chocolate, red wine and green tea contains considerable amount of catechin molecule. They can combine to form gallocatechin, epicatechin gallate and epigallo-catechin gallate in leguminous plant, grape and mainly in tea. (Manach et al., 2004; Peterson & Dwyer, 1998).

1.2 Onion and onion skin

Onion (*Allium cepa*) is one of the most consumed vegatable in the world. Its classification is based on its color (yellow, red or white), dry matter content and bitterness. Flesh parts of onion are widely used in human diet. Since onion contains various kinds of phytochemicals, it has health promoting effects like antioxidant, anti-inflammatory, anti-cancer and antimicrobial effects (Albishi, John, Al-Khalifa, & Shahidi, 2013).

The composition of onion is variable and it depends on its maturation, habitat, storage time and cultivar (Abayomi & Terry, 2009). It includes water (89.1%), carbohydrates (9.3%), protein (1.1%), fat (0.1%), vitamins

and minerals (Nile & Park, 2013). The flavor of the onion is due to sulphur containing compounds which are structured by the cleavege of the sulphoxides (Griffiths, Trueman, Crowther, Thomas, & Smith, 2002). In addition, onion shows considerable amount of total dietary fiber and good soluble to insoluble dietary fiber ratio which are directly related to the physical and metabolic effects. There are two main chemical groups: flavonoids and the alkenyl cysteine sulphoxides. Onion flavonoids have two subgroups which are flavonols such as quercetin, myricetin and kaempferol and anthocyanins which gives the red/purple color (Benítez et al., 2011; Griffiths et al., 2002).

In industrial scale, onion has large amount of wastes which are onion skin, roots or damaged bulbs. These wastes present some environmental problems due to onions' characteristic aroma from sulphur containing compounds. Since wastes develop rapid growth of phytopathogenic agents like *Sclerotium cepivorum*, they are not suitable for the animal feed or landfill suppression (Roldán, Sánchez-Moreno, Ancos, & Cano, 2008). Thus, processing and utilization of the wastes can be a possible solution. Recent studies show that sulfhydryl groups can be used for the inhibition of the polyphenol oxidase enzyme which causes the enzymatic browning of fruits and vegetables and can affect the organoleptic and visual characteristic of products negatively (Kim, Kim, & Park, 2005).

Moreover, flavonoid levels in the edible part is lower than flavonoid level of onion skin which is about 2-10 g/kg. Onion skin contains mostly glycosides of quercetin derivatives like quercetin diglucoside and quercetin acyclone. This components provide antioxidant and radical scavening acitivity to superoxide radical (Albishi et al., 2013; Suh, Lee, Cho, Kim, & Chung, 1999).

1.3 Microencapsulation

Encapsulation is a process in which one material is entrapped or coated with another material in order to protect the coated material against adverse conditions and the nutritional deterioration. The coated one is named as core or active material and surrounding one is coating material (Mcnamee, O'Riordan, & O'Sullivan, 1998). Encapsulation technique is commonly used in food, pharmaceutical and cosmetic industry in order to protect fragile or unstable compound from surrounding environment, to control the release of core material and to mask the undesired properties of core material (Dubey, Shami, & Rao, 2009).

Microcapsules are categorised by their size or morphology. In terms of size, there are micro capsules whose size ranges from 1 micron to few mm values and nanocapsules whose diameter is in nanometer range (Dubey et al., 2009). With respect to morphology, microcapsules can be classified into three classes which are monocore, polycore and matrix (Figure 1.2). Monocore microcapsules contain a single chamber around the core, on the other hand in polycore capsules, there are different size of cores enclosed within the shell. In matrix encapsulation, the homogenous distribution of core material is seen in the shell material (Umer, Nigam, Tamboli, & Nainar, 2011).

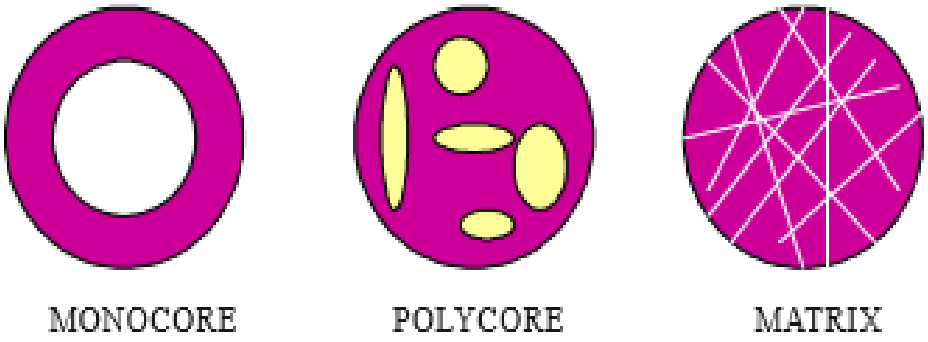


Figure 1.2 Morphology of different kinds of capsules (Dubey et al., 2009)

1.3.1 Homogenization techniques used in encapsulation process

For the encapsulation of active component with an aqueous solution, homogenization for emulsion preparation is the first part of the encapsulation process. During homogenization, energy density is important parameter for higher encapsulation efficiency values (McClements, 2005). Low energy emulsification method by high-speed homogenizer and high energy emulsification methods by ultrasonication and microfluidization can be used for homogenization (Jafari, He, & Bhandari, 2007).

1.3.1.1 High-speed homogenizer

High-speed homogenizers are the most common and cost-effective way for homogenizing the mixtures of oil and aqueous phase in food industry. It is a batch process that contains an impeller and a stator made of stainless steel.

The rotating head, up to 3600 rev/min, generates rotational, horizontal, vertical, longitudinal and radial velocity component in mixture (McClements, 2005). Figure 1.3 shows the schematic representation of high-speed homogenizer. Since during blending, there might be a slight increase in temperature due to viscous dissipation, the temperature control is necessary for heat sensitive materials like phenolic compounds. The droplet size which ranges from 2 and 10 μm in diameter is decreased by high- speed homogenizer. As rotation speed and homogenization time increase, the particle size approaches to lower limit (Shirgaonkar, Lothe, & Pandit, 1998; McClements, 2005).

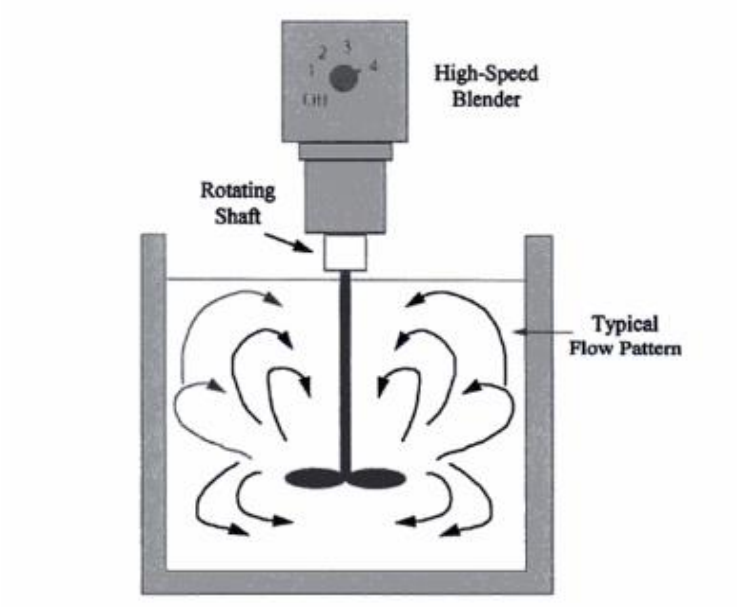


Figure 1.3 Schematic representation of high-speed homogenizer (McClements, 2005)

High-speed homogenizers are used prior to other homogenization techniques for efficient emulsion stability and desired particle size. In a recent study, it was found that in order to get more tiny droplet size, along with the high-speed homogenizer, the high-pressure homogenizer should be applied with its additional shear force (Karthik & Anandharamakrishnan, 2016).

1.3.1.2 Ultrasonication

Ultrasonic emulsification is a dispersing technique that was initially applied for the solid-liquid nanodispersion. Ultrasonic homogenizer uses high-intensity ultrasonic waves with frequencies greater than 20 kHz. These waves produce high pressure and shear gradient which cause distruption of droplet by turbulent effect and cavitation mechanims (McClements, 2005).

The most commonly used methods for ultrasonication are piezoelectric transducers which are used for small volumes of samples and liquid jet generators which are mainly for industrial applications (McClements, 2005). The ultrasonic homogenizer process parameters are frequency or wave intesity, sonication time, hydrostatic pressure, viscosity and temperature (He, Bhandari, & Jafari, 2016).

The working principle of the ultrasound is cavitation. Jafari, He, & Bhandari (2016) explained cavitation as is the formation and the collapse of vapor cavities in liquid mixture. Due to local velocity change, the local pressure is reduced to vapor pressure of the flowing liquid. Then by the help of the collapse of cavities ultrasonic wave radiate along the solution and break the dispersed liquid (He et al., 2016). Two steps are proposed for ultrasonic

homogenization. At first step, dispersed phase erupts into continuous phase, while at second step, by cavitation process droplets are broken up into small sizes. Very small droplet size like 500 nm is achieved (He et al., 2016; Jafari et al., 2007).

There are many studies for the comparison of ultrasonication with other homogenization techniques. The microbial cell distruption with ultrasonic homogenization and high-speed homogenizer was compared with each other. Ultrasonic technique is found more efficient due to cavitation mechanism (Shirgaonkar et al., 1998). Before the ultrasonication process, the homogenization with high-speed homogenizer gives better efficiency results for the encapsulation of sour cherry pomace (Cilek, Sahin, Sumnu, Luca, & Hasirci, 2012) For the heat stability effectiveness of whey protein solutions homogenization techniques were compared. The results showed that ultrasonication was equally effective with other high shear techniques due to acoustic cavitation (Koh et al., 2014). Jafari, et al. (2016) investigated the encapsulation efficiency of d-limonene with sonication and microfluidization methods. Ultrasonication was found to be an easy way to obtain capsules compared to microfluidization.

1.3.1.3 Microfluidization

Though microfluidization is commonly used in pharmaceutical industry for many years, in recent years this technique has also been used in food industry especially in dairy products (He et al., 2016). As shown in the Figure 1.4, this device consists of three parts: a fluid inlet, a pumping device and an interaction chamber which contains two channels one for flow

and the other for interaction of fluids. When fluids enter the system, by the help of pumping device which can reach high pressures up to 275 MPa, they are accelerated with high velocity and crashed with each other, so droplets are disturbed (McClements, 2005).

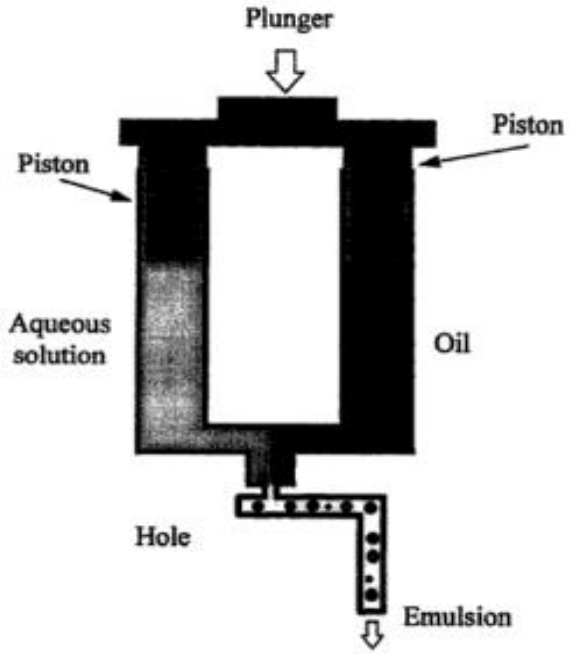


Figure 1.4 Schematic representation of microfluidizer (McClements, 2005)

Several studies showed that microfluidization was superior to other techniques in terms of producing smaller particle size distribution. In addition to reaching high shear value, the cavitation helped to reduce particle size. The mean particle size of emulsions with microfluidizer ranges from 5 μm to 50 nm (Grumezcu, 2016). In some studies microfluidization was compared with the ultrasonication. Although, the efficiency values are

more higher in microfluidizer, it is less practical because of the temperature increase during pressure increase, in addition the overprocessing occurs in microfluidizations (Abismail, Canselier, Wilhelm, Delmas, & Gourdon, 1999; Bouaouina, Desrumaux, Loisel, & Legrand, 2006; He et al., 2016).

1.3.2 Coating materials used in encapsulation process

The encapsulant formulation has a significant role in encapsulation process. For successful encapsulation, thermal or mechanical stability of core materials to some external factors (e.g., pH, chemicals) is important (Mcnamee et al., 1998). Ghosh (2006) highlighted that the convenience of core and coating material can enhance the encapsulation efficiency. There are different kinds of coating materials used for encapsulation process which are carbohydrate based, protein based and lipid based coating materials.

1.3.2.1 Carbohydrate based coating materials

Carbohydrates are commonly used as a coating material as encapsulant. There are several kinds of carbohydrates such as starches, corn syrup solids and gums. They can be in form of homopolymer that contain a single type of repeating unit or copolymer that is composed of the mixture of repeating units. These polymers contain sugar residues and their derivatives (Zuidam & Nedović, 2010). The diversity, attainability, film forming ability and low cost properties make these carbohydrates the favored alternative for encapsulation (Gharsallaoui, Chambin, Roudaut, Voilley, & Saurel, 2007)

Maltodextrins are produced from the partial enzymatic hydrolysis of starch. Dextrose equivalence (DE) is the indication of starch hydrolysis (Parikh, Agarwal, & Raut, 2014). It is shown that Maltodextrin with DE value of 4.0-7.0 can protect phenolic compounds with higher efficiency values than Maltodextrin DE value of 18.5 (Laine, Kylli, Heinonen, & Jouppila, 2008). The effect of Maltodextrin molecules with different DE values were investigated on microencapsulation of the anthocyanin pigments of black carrot (Ersus & Yurdagel, 2007). In addition, maltodextrin has good solubility in water and low viscosity values even at high concentrations. These properties make maltodextrin useful for coating material. On the other hand, maltodextrins are deficient in terms of emulsification property and lack of surface-active features. For this reason, combining of other coating materials with maltodextrin is required to form stable capsules (Rosenberg & Sheu, 1995).

Gum arabic (Gum Acacia) is composed of branched arrangement of simple sugars like galactose, glucuronic acid, arabinose and rhamnose and small amount of covalently bonded protein. This protein gives functional properties to gum arabic (Mcnamee et al., 1998). Its source is the natural leakage from acacia trees. Since it has high water solubility, low viscosity than other gum types and ability of good retention of volatile compounds, it is mostly suitable for flavor encapsulation (Madene, Jacquot, Scher, & Desobry, 2006). In addition, it can create a protective film around oil droplets and acts like emulsifier. In other words, it prevents aggregation by forming a thick layer (Zuidam & Nedović, 2010). On the other hand, because of the high cost and availability, the usage of gum arabic is limited for encapsulation process. The combination is the alternative way for using Gum Arabic. It was shown that the Gum Arabic/Maltodextrin combination

was more efficient than only maltodextrin in encapsulation of oil (Carneiro, Tonon, Grosso, & Hubinger, 2013). Moreover, the encapsulation of sour cherry pomace with maltodextrin and maltodextrin-gum arabic combination were tested. Results showed that the efficiency values are higher in combination coating than only maltodextrin containing (Cilek et al., 2012).

1.3.2.2 Protein based coating materials

Proteins are macromolecules that are composed of series of amino acids. There are several types of proteins which are derived from animal origin (gelatin, whey protein or casein) or plant origin (soy, wheat or pea) (Mishra, 2016). They are widely used for encapsulation of active substances since they have different chemical groups with amphiphilic properties that can correlate with interaction of various chemical groups (Madene et al., 2006). Proteins have some functional properties such as good solubility in water, high film forming and emulsification, flexibility of molecular chain, viscosity; thus they can stabilize the emulsion during process (Madene & Jacquot, 2006; Mishra, 2016).

Casein is the most dominating phosphoprotein in milk. It contains four main components: α_1-casein , α_2-casein, β-casein, κ-casein and one minor component γ-casein. Each of them can be in various proportions from milk to milk due to genetic variations in cow. Since cysteine amino acid is low in casein, the disulfide linkage is few and casein has random coil form (C.Huber, 2009). Casein is a heat stable protein that do not denature easily. Thus, for protection of core material, casein is good option as coating material (Mishra, 2016). Due to its amphiphilic character, higher emulsification properties of casein is an effective encapsulating material for

lipid containing emulsions (Hogan, Mcnamee, O'Riordan, & O'Sullivan, 2001). It allows better homogenous distribution around fat drop (Gharsallaoui et al., 2007). In recent research, curcumin was encapsulated with milk protein sodium caseinate molecule. The effect of different processing conditions like pH change, temperature variation and ionic strength were investigated and the most stable emulsion was specified (Kumar et al., 2016).

Whey protein is a valuable product obtained from cheese manufacture and is used commonly as a coating material. β-lactoglobulin (major protein), α-lactalbumin, bovine serum albumin and immunoglobulins are whey proteins (Panaras, Moatsou, Yanniotis, & Mandala, 2011). There are two kinds of whey protein type: whey protein concentrate (WPC) and whey protein isolate (WPI). In WPC lactose, water and some minerals are removed and approximate protein concentration ranges from 30% to 80%. In WPI, the protein concentration is higher than 90% (Perez-Gago, Serra, & Río, 2006). Because of having small molecular weight with its spherical shape, whey protein shows low viscosity in high concentration. It is amphiphilic and has high solubility. In addition, with high surface activity, whey protein acts as film-forming and emulsifiying agent (Rosenberg & Sheu, 1995). There was a research that investigated the encapsulation of oregano essential oil and aroma extract of citronella with whey protein concentrate and skimmilk powder (Baranauskiene, Venskutonis, Dewettinck, & Verhé, 2006).

1.3.2.3 Lipid based coating materials

Lipid based materials are hydrophobic i.e. insoluble in water. Lipids has large diversity involving fatty acids and alcohols, glycerols, phospholipids and waxes which are the esters of fatty acid (Zuidam & Nedović, 2010).

Fatty alcohols can act as non-ionic surfactant and has emulsifying properties like mono and diglycerides. Since, phospholipids has amphiphilic character, for the encapsulation process they are most appropriate and have extensive usage as coating material (Mishra, 2016).

In one research, it was underlined that the lipid addition to starch based coatings had positive effect on the barrier properties of microcapsules. Due to hydrophobicity characteristic of lipid molecules gas permeability was significantly decrease. By decreasing oxygen transition, oxidation sensitive materials was permanent within the capsule without degredation (Garcia, Martino, & Zaritzky, 2000). Moreover, in order to increase the bioaccesibility curcumin, the encapsulation procedure was applied with different lipid molecules like short, medium, and long chain triacylglycerols (Ahmed, Li, Mcclements, & Xiao, 2012)

1.3.3 Drying methods used in encapsulation

For preparing of microcapsules, spray drying and freeze drying are the most frequently used drying methods.

1.3.3.1 Spray drying

Spray drying is a low-cost process that is commonly used for the encapsulation of flavors , volatiles , fragrances and oils (Ghosh, 2006). However, spray drying is not generally suitable for heat sensitive materials like phenolic compounds (Patel, Suthar, & Patel, 2009). Schematic illustration of the spray drying process is seen in Figure 1.5. The method of

spray drying contains four main steps: (1) preparation of the feed solution, (2) atomization, (3) solvent evaporation, (4) recovery of encapsulated powder. Atomization is the most important step in which the maximum heat and mass transfer surface can be optimised between hot dry air and liquid mixture. The optimisation is done with feed temperature, inlet hot air temperature and feed properties such as inlet flow rate or viscosity. The evaporation can be achieved by co-current or counter-current hot air (Gharsallaoui et al., 2007).

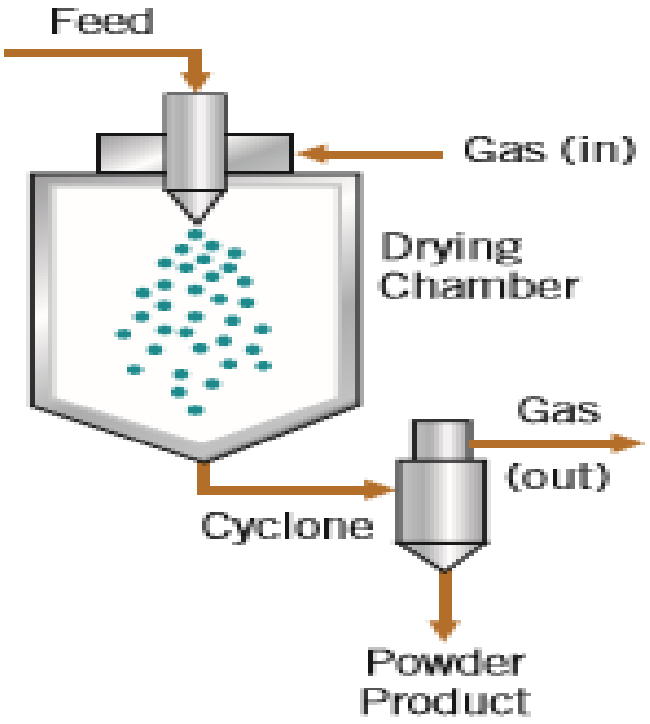


Figure 1.5 Schematic illustration of spray dryer (Ghosh, 2006)

One drawback of spray drying is that during process some low-boiling point aromatics may be lost. Since the core material can be on the surface of the capsule, by high temperature oxidation and some flavour changes are possible. The other problem of the spray drying is producing very narrow size distributed powder which needs further processing like agglomeration (Gharsallaoui et al., 2007; Madene et al., 2006).

1.3.3.2 Freeze Drying

The freeze-drying technique, which is also known as lyophilization or cryodesiccation, is widespread method for drying of heat sensitive materials such as phenolic and antioxidant, tocopherols, carotenoids, ascorbic acid containing substances. Antioxidant activity of selected tropical fruits were investigated in terms of freeze drying and it was shown that there was no significant change in antioxidant activity (Shofian et al., 2011). The drying occurs in two step: (1) primary drying: sublimation of frozen solvent from capsules and (2) secondary drying: with low pressure the desorption of solvent from mixture (Mujumdar, 2007). In the sublimation step, unbound solvent is sublimated, on the other hand in secondary drying, remaining bound solvent is gone away by vacuum (Mujumdar, 2007). Before freeze drying process the sample should be frozen. Due to pre-freezing process, freeze drying preserve the shape of microcapsule (Nagata, 1996).

Freeze dryer are commonly used for encapsulation process. The rosemary essential oil was encapsulated with maltodextrin and whey protein concentrate by using freeze dryer (Turasan, Sumnu, & Sahin, 2015). In addition, there was a research about the encapsulation of red wine polyphenols with freeze drying procedure (Sanchez, Baeza, Galmarini, Zamora, & Chirife, 2013).

Though freeze drying can be used for several applications in food or pharmaceutical industry, because of high cost value and time-consuming process, among the other drying techniques, it is less used. It has low commercial availability for huge amounts (Madene et al., 2006).

1.4 Objectives of the study

In recent years, diseases such as high blood pressure, diabetes and obesity have reached to epidemic levels. Since foods containing phenolic and antioxidant compounds help the prevention of this kind of diseases, people are willing to consume more functional foods derived from the natural supplementary substances (such as fruits and vegatables). Onion is one of the functional food with its antimicrobial, anticancer, antioxidant properties and high proportion of phenolic substances. People consume onion very often in different ways, however onion skin is always thrown away. Studies showed that onion skin has significant amount of antioxidant and phenolic substances. For this reason; onion skin can be recommended to be consumed. In addition, onion skin has high amount of color pigments coming from phenolic substances. Instead of discarding of onion skin, this color pigments can be used in cosmetic industry.

As onion skin can not be consumed directly, the encapsulation method can be used to benefit from its functionality. By microencapsulation, the bioactive compounds are completely isolated from the external factors. In this technique, also the unwanted odor and taste of the ingredient are masked and the loss of the beneficial and susceptible materials such as, antioxidants and phenolics are prevented.

The objective of this study was to encapsulate phenolic compounds extracted from onion skin. Moreover, the effects of core to coating ratio and different coating materials were investigated in terms of encapsulation efficiency, total phenolic content, antioxidant activity, morphology and particle size distribution. In addition, heat stability of microcapsules were also studied.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Onion skin was taken from the market. Firstly, in order to get rid of the foreign materials, skin was washed with cold water, then spread out to wide space for nearly 3 or 4 days to completely dry out. Dried onion skin was stored at 25 °C in polyethylene bags.

Maltodextrin whose Dextrose Equivalent (DE) value was 4.0-7.0, casein sodium salt from bovine milk and Gum Arabic (acacia powder) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Whey protein concentrate (WPC) whose protein content was 80%, was taken from Göktürk (Turkey, URL1). They were used as the coating materials of capsules.

The reagents used in the experiments, which were acetic acid, ethanol, methanol, gallic acid, sodium carbonate, $DPPH^{\cdot}$ (2,2-diphenyl-1-picrylhydrazyl), Folin-Ciocalteau's phenol reagent were all bought from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2 Grinding of onion skin

Prewashed, dried and packaged onion skins were ground by a grinder (Pulverisette 16, Fritsch GmbH Milling and Sizing, Germany). Sieve opening of the grinder was 1mm.

2.3 Extraction of phenolic compounds

In order to extract phenolic compounds, 20 g of ground onion skin was weighted and mixed with 400 ml of 50:50 v/v ethanol and water. They were mixed in the 500 ml glass erlenmayer flask glass. The extraction was done in shaking water bath (GFL 1086, Burgwedel, Germany) at 40 °C and 70 rpm for 4 hours. After 4 hours, aqueous part was separated from solid part by using filter cloth. Then, the extract was vacuum filtered using micro filter paper (Whatman 4, GE Healthcare UK Limited).

2.4 Phenolic powder preparation

The filtered extract was concentrated at 35°C by means of rotary vacuum evaporator (Heidolph Laborota 4000 efficient, Schwabach, Germany). The concentrated extracts were poured to glass petri dishes and were frozen for 1 day. Then samples were dried by freeze dryer (Christ, Alpha 1-2 LD plus, Osterode, Germany). Drying was performed below 0.1 mPa for 48 hours. At the end of drying, onion skin phenolic powder was ground to fine powder form, and stored at freezer at -18°C until it was used.

2.5 Preparation of coating materials for the microcapsules

Maltodextrin (MD) was mixed with Gum Arabic (GA), casein sodium salt from bovine milk (Casein) or Whey Protein Concentrate (WPC) at different ratios (10:0, 8:2, 6:4). Total solid content of coating mixtures were 10%. MD with DE value of 4.0-7.0 was used for encapsulation, since it shows higher encapsulation efficiency value and can protect phenolic compounds better than Maltodextrin with DE value of 18.5 (Laine et al., 2008). MD solutions were mixed with high speed homogenizer at 7000 rpm for 3 min. In addition, 4% and 8% (w/w) GA, 4% and 8% (w/w) Casein and 4% and 8% (w/w) WPC solutions were prepared with again high speed homogenizer at 6000 rpm for 4 min. Coating solutions were prepared 1 day before the encapsulation process to obtain full hydration and they were stored in refrigerator at 4°C.

2.6 Encapsulation

Dried phenolic powder was encapsulated with four different coating material types (Maltodextrin only, Maltodextrin:Gum Arabic, Maltodextrin:Casein, Maltodextrin:WPC) at three different ratios which were 10:0, 8:2, 6:4. In addition, two different core to coating ratios (1:10 and 1:20) were tried.

Phenolic powder of onion skin of 2 g and 1 g were separately weighed via precision balance for 1:10 and 1:20 core to coating ratio respectively. Then, 20 g of required coating material was added into it. In order to get capsules, the mixtures were homogenised by a high-speed homogenizer (IKA T25 digital Ultra-Turrax, Selangor, Malaysia) at 10000 rpm for 10 min. Samples

were placed in ice bath to prevent damaging of phenolic compounds due to overheating. Then, capsules were freeze dried for 48 hours. Each experiment was replicated twice.

2.7 Analysis of phenolic powder and encapsulated phenolic powder

2.7.1 Determination of total phenolic content of capsules

The total phenolic content (TPC) was determined by Folin-Ciocalteau method (Beretta, Granata, Ferrero, Orioli, & Maffei, 2005). In this method, in the presence of phenolic substances, Folin-Ciocalteau reagent was reduced with sodium carbonate and as a result of this reduction, color change was observed (Beretta et al., 2005).

100 mg of phenolic powder was accurately weighed and mixed with 1 ml of ethanol:water (50:50 v/v) using a Vortex (ZX3, VELP Scientifica, Usmate, MB, Italy) for 1 min. Then, it was filtered through a micro filter (0.45 μm Gema Medical Filter, Spain). The same procedure was applied to encapsulated phenolic powder, however 100 mg of encapsulated phenolic powder was dissolved in 1 mL of ethanol:acetic acid:water mixture (50:8:42 v/v) instead of ethanol : water (50:50 v/v) solution and then filtered.

For TPC determination, 2.5 mL of 0.2 N Folin-Ciocalteau (2N, SIGMAALDRICH F9252) reagent and 500 μl diluted sample were mixed in tubes with Vortex for 5 sec. Mixture was kept in dark place for 5 min, then 2 ml of sodium carbonate solution (75g/L, SIGMAALDRICH S7795) was added to the tubes and mixed again by Vortex for 5 sec. All samples were

placed in dark at 25°C for 1 hour. Then, by using UV/VIS spectrometer T 70, (PG Instruments LTD, UK) the absorption values were recorded at 760 nm. Blank was the mixture of 2.5 mL of 0.2 N Folin-Ciocalteau and 2 ml of sodium carbonate solution (75 g/L).

Calibration curve was prepared with gallic acid solution at different concentrations (20, 40, 60, 80, 100 ppm in ethanol:water (50:50 v/v) mixture for dried phenolic powder and in ethanol:acetic acid:water mixture (50:8:42 v/v) for dried encapsulated phenolic powder). By using calibration curve, total phenolic content was expressed as mg gallic acid equivalents (GAE) / g dry weight.

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

2.7.2 Determination of surface phenolic content of capsules

In order to determine the surface phenolic content (SPC) of capsules, Folin-Ciocalteau method was used like TPC content determination described in Section 2.7.1 (Saénz, Tapia, Chávez, & Robert, 2009). The only difference was mixing of 100 mg of phenolic powder microcapsules with 1 mL of ethanol and methanol mixture (50:50 v/v).

Calibration curve with ethanol: methanol (50:50 v/v) mixture was prepared with gallic acid solutions at different concentrations (10, 20, 30, 40, 50 ppm). The SPC of phenolic powder was expressed as mg gallic acid equivalents (GAE)/ g dry weight.

Calibration curve is given in Figure A.3.

2.7.3 Encapsulation efficiency

The encapsulation efficiency (EE) was calculated using Equation (2.1) (Cilek et al., 2012);

$$EE(\%) = \frac{EPC}{TPC} \times 100 = \frac{TPC-SPC}{TPC} \times 100 \quad (2.1)$$

where EPC was the encapsulated phenolic content which was calculated by substracting surface phenolic content (SPC) from total phenolic content (TPC). Surface phenolic content (SPC) is the phenolic content that could not be encapsulated.

2.7.4 Total antioxidant activity with DPPH˙ radical scavenging method

For determining total antioxidant activity (AA), DPPH˙ (2,2-Diphenyl-1-picrylhydrazyl) method was used (Yen & Duht, 1994). Since DPPH˙ is very sensitive substance that can be affected from heat and light, its degradation occurs very rapidly. It is always kept in dark place and in refrigerator. If the substance has antioxidant characteristics, DPPH˙ solution changes color and by the help of spectrophotometer this change can be determined.

In the case of both dried phenolic powder and capsules, 100 mg of sample was weighed and mixed with 1 mL ethanol:acetic acid:water mixture (50:8:42 v/v) using Vortex (ZX3, VELP Scientifica, Usmate, MB, Italy) for 1 min. The mixture in the tube settled and the upper liquid part of emulsion was drawn into syringe and filtered through a filter whose pore size is 0.45 µm (Gema Medical Filter, Spain). Then, samples were diluted with ethanol:acetic acid:water mixture (50:8:42 v/v). Pure methanol was used as a blank. 100 µl methanol and 3.9 ml of 25 ppm DPPH˙ solution (2.5 mg DPPH˙/100 ml methanol) was mixed with Vortex for 5 sec and absorbance

was measured by UV/VIS spectrophotometer T 70 (PG Instruments LTD, UK) at 517 nm. This value was recorded as A_1. Diluted sample of 100 µl and 3.9 ml of 25 ppm DPPH˙ solution (2.5 mg DPPH/100 ml methanol) were mixed with Vortex for 5 sec and kept in dark for 1 hour at 25°C. After 1 hour, the reaction of DPPH solution with samples was completed and the absorption values were recorded by UV/VIS spectrophotometer T 70 (PG Instruments LTD, UK). This value was recorded as A_2 .

A_1 and A_2 values can be converted to concentrations of C_1 and C_2 by calibration curve. Calibration curve was prepared with different concentrations of DPPH˙ (5, 10, 15, 20, 25 ppm) in methanol. Then, by using concentrations of C_1 and C_2, the result was evaluated with Equation (2.2),

$$\text{AA (mg DPPH}^{\cdot}\text{/ g dry weight)} = \frac{(C_1 - C_2)}{W} \times \text{D} \times \text{V} \qquad (2.2)$$

where V is the volume of extract in mL, D is the dilution rate, W is the amount of dry sample in g.

Calibration curve is given in Figure A.4.

2.8 Particle size analysis

Particle size analysis of emulsions with different coating material combinations and core to coating ratios were performed by using particle size analyser (Mastersizer 2000, Malvern Instruments, Worcestershire, UK). The size of the particles were analysed by the laser diffraction technique and size distributions were specified as volume percentage versus capsule diameter. Sauter mean diameter D32 in µm, span and specific surface area

in m^2/g values were calculated by the instrument. All measurements were done in duplicates.

The mean particle size of capsules was expressed as Sauter mean diameter D32 in µm (Equation (2.3)). The span value gives the width of capsules in diffusion and expressed in Equation (2.4) (McClements, 2005).

$$D32 = \frac{\sum n_i \ d_i^3}{\sum n_i \ d_i^2} \quad (2.3)$$

$$\text{Span} = \frac{[d(v,90) - d(v,10)]}{d(v,50)} \quad (2.4)$$

Where, d_i is the diameter and n_i is the number of particles in each size and d(v,90), d(v,50), d(v,10) are the diameter values at 90%, 50% and 10% of the cumulative volume, respectively; i.e. d(v,50) is the median diameter and d(v,90)-d(v,10) is the range of data.

2.9 Morphological characteristics of microcapsules

To analyze the differences between morphological characteristics of microcapsules, the samples were analysed under light microscope. Samples were spreaded as a very thin layer on glass microscope lamelle and were viewed under the light microscope (Primo Vert, Zeiss, Jena, Germany). The ToupView Software was used to analyse the samples. The microscope images were taken by microscopic camera (Sony CCD Color Digital Video C-Mount Microscope Camera, Tokyo, Japan). Microcapsules prepared with Maltodextrin (10:0 w/w) only, Maltodextrin:Gum Arabic (8:2 w/w), Maltodextrin:Casein (8:2 w/w) and Maltodextrin:WPC (8:2 w/w) and with core to coating ratio of 1:10 and 1:20 were analysed for their morphological characteristics at magnificion of 40× .

2.10 Heat stability of microcapsules

Phenolic substances and antioxidants are easily degradable materials to environmental changes such as, temperature variation, light, pH changes. For this reason, encapsulation procedure is applied in order to stabilize the fragile substances. The stability of microcapsules to extreme conditions has significant role.

The heat stability of microcapsules was evaluated at 80°C by using water bath. The temperature was controlled with thermometer regularly to stabilize 80°C. The antioxidant content was evaluated by DPPH˙ method and phenolic content measurements were done by Folin-Ciocalteau method (Saénz et al., 2009; Yen & Duht, 1994). The procedure was described in section 2.7.2 and 2.7.4. Total phenolic content of phenolic powder without coating extracted from onion skin and microcapsules prepared with different coating material combinations of MD:Casein and MD:WPC at 1:20 core to coating ratio were measured. The heat stability analysis was performed by keeping samples at 80°C for 1 hr, 2 hr, 3 hr, 3.5 hr and 4 hr. In addition, the antioxidant activity was evaluated for capsules prepared with maltodextrin: casein and maltodextrin: WPC combination at 8:2 ratio. Each experiment was replicated twice.

2.11 Statistical analysis

The independent variables used in the study were core to coating ratio, coating material type and ratios. In order to decide if there were significant difference between these variables on dependent variables (total phenolic content, surface phenolic content, antioxidant activity, encapsulation efficiency, particle size analysis, heat stability), the analysis of variance (ANOVA) was applied by MINITAB (Version 16). Tukey's Multiple Comparison Test was used for comparisons ($p \leq 0.05$). All results were replicated twice for each variable.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Surface phenolic content, total phenolic content and encapsulation efficiency of microcapsules

Table 3.1 showed the effect of coating material types and core to coating ratios on total phenolic content, surface phenolic content. ANOVA results showed that there was significant ($p \leq 0.05$) differences between coating type and core to coating ratio in terms of total phenolic content, surface phenolic content (Table B.1 & Table B.2).

Table 3.1 Total phenolic content and surface phenolic content of capsules having different coating material type, coating ratio and core to coating ratio

Coating type	Coating ratio	Core: Coating ratio	Total phenolic content (mg GAE/ g dry weight)	Surface phenolic content (mg GAE/ g dry weight)
MD	10:0	1:20	209.24±1.20^{e*}	72.86±0.89^{g*}
MD:GA	8:2	1:20	253.05±1.31^{d}	112.25±1.00^{cd}
MD:GA	6:4	1:20	259.73±0.93^{d}	104.68±0.92^{de}
MD:Casein	8:2	1:20	161.36±1.38^{g}	25.21±1.43^{j}
MD:Casein	6:4	1:20	183.80±1.09^{f}	19.95±0.37^{j}
MD:WPC	8:2	1:20	183.34±1.40^{f}	38.02±1.05^{i}
MD:WPC	6:4	1:20	189.55±1.21^{f}	57.18±1.18^{h}
MD	10:0	1:10	328.46±1.41^{a}	119.04±1.00^{bc}
MD:GA	8:2	1:10	300.25±1.28^{b}	123.55±1.51^{b}
MD:GA	6:4	1:10	297.28±1.20^{b}	141.04±1.59^{a}
MD:Casein	8:2	1:10	261.54±1.67^{d}	72.15±0.64^{g}
MD:Casein	6:4	1:10	260.22±1.74^{d}	85.44±2.19^{f}
MD:WPC	8:2	1:10	282.55±1.37^{c}	97.12±1.37^{e}
MD:WPC	6:4	1:10	261.79±1.25^{d}	111.90±1.47^{cd}
Phenolic powder			510.871±4.04	

*Different letters within the same column shows significant difference ($p \leq 0.05$)

According to Table 3.1 total phenolic content of microcapsules were lower than phenolic powder without coating extracted from onion skin. This might be due to the loss of phenolic compounds during encapsulation. Phenolic powder was encapsulated by using a high speed homogenizer. Homogenization step could distrupt the interfaces of phenolic powder and coating material by external mechanical force and resulted in homogenous distribution of phenolic powder into coating material. This mechanical force might give mechanical damage to phenolic compounds (McClements, 2005). Another reason for loss of the total phenolic compounds could be explained by the effect of freezing. Since homogenized microcapsules were freeze dried, during freezing onion skin matrix might be distrupted which might cause liberation of enzymes. The increased activity of enzymes upon thawing might have resulted in degration of phenolic compounds (Shofian et al., 2011). The same result was obtained in various studies in which the effect of freeze drying on total phenolic content was investigated (Franceschinis, Salvatori, Sosa, & Schebor, 2014; Kuck, Pelayo, & Noreña, 2016; Shofian et al., 2011). Since phenolic components are susceptible to external factors, the degradation of phenolic compounds are possible during the encapsulation process (Cavalcanti et al., 2011; Fang & Bhandari, 2010).

Total phenolic content of microcapsules ranged between 161.36 mg GAE/g dry weight and 328.46 mg GAE/g dry weight. Because of the higher phenolic powder concentration in microcapsules with 1:10 core to coating ratio, total phenolic content of this capsule were higher than capsules with 1:20 core to coating ratio. The total phenolic content of microcapsules coated with only maltodextrin and with maltodextrin gum arabic combination were found to be higher compared to maltodextrin and protein based coating material combinations. In other words; the loss of total phenolic compounds during encapsulation process were lower in the

capsules without protein. Maltodextrin can protect encapsulated material from oxidation when combined with gum arabic. This combination was useful due to emulsifying ability of gum arabic whose structure was highly branched with several kinds of sugar arrangements (Cilek et al., 2012; Mcnamee et al., 1998). However, coating materials containing carbohydrate and protein combination could not protect phenolic compounds as much as maltodextrin and gum arabic combination. Protein based coating materials were found to be more effective in coating of oily core materials such as essentials oils. The amphiphilic characteristics of protein molecules can form film on the oily core material and lead to strong interaction between water and oil surfaces (Hogan et al., 2001; Turasan et al., 2015). Maltodextrin-casein and maltodextrin-WPC combination did not show significant difference in terms of total phenolic content values (Table 3.1).

Table 3.2 Encapsulation efficiency values of capsules having different coating type, coating ratio and core to coating ratio

Coating type	Coating ratio	Core: Coating ratio	Encapsulation efficiency
MD	10:0	1:20	$65.17 \pm 0.86^{de*}$
MD:GA	8:2	1:20	55.64 ± 0.87^{gh}
MD:GA	6:4	1:20	59.69 ± 0.39^{fg}
MD:Casein	8:2	1:20	84.39 ± 2.82^{ab}
MD:Casein	6:4	1:20	89.15 ± 0.02^{a}
MD:WPC	8:2	1:20	79.29 ± 1.17^{b}
MD:WPC	6:4	1:20	69.83 ± 0.26^{cd}
MD	10:0	1:10	63.76 ± 0.22^{ef}
MD:GA	8:2	1:10	58.85 ± 0.59^{fg}
MD:GA	6:4	1:10	52.55 ± 0.22^{h}
MD:Casein	8:2	1:10	72.41 ± 0.19^{c}
MD:Casein	6:4	1:10	67.16 ± 0.08^{cde}
MD:WPC	8:2	1:10	65.63 ± 0.34^{de}
MD:WPC	6:4	1:10	57.25 ± 1.17^{gh}

*Different letters within the same column shows significant difference ($p \leq 0.05$)

Encapsulation efficiency is the most important criteria to encapsulate core material properly. Less amount of phenolic content on the surface of microcapsules shows more efficient encapsulation process. The efficiency values of microcapsules with core to coating ratio of 1:20 changed between 55.64 and 89.15%, while the efficiency values were between 52.55 and 72.41% for microcapsules with core to coating ratio of 1:10. As can be seen in Table 3.2, the efficiency values were higher in capsules with core to coating ratio of 1:20 (Table B.3). Since the concentration of phenolic powder without coating was higher in capsules with 1:10 core to coating ratio, coating material was not enough to envelop the core material as compared to capsules with core to coating ratio of 1:20. As core to coating ratio changed from 1:20 to 1:10, the encapsulation efficiency decreased in literature (Cilek et al., 2012; Turasan et al., 2015; Yazicioglu, Sahin, & Sumnu, 2015). Coating material ratio had also significant effect on encapsulation efficiency (Figure 3.1-3.3). Significant difference was determined between 10:0, 6:4 and 8:2 ratios in terms of encapsulation efficiency ($p \leq 0.05$).

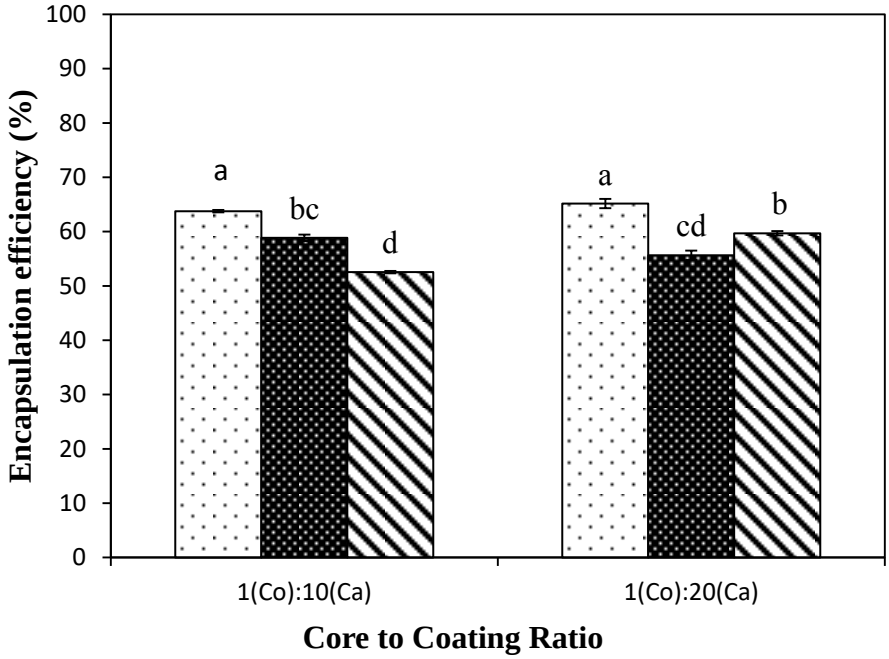


Figure 3.1 The encapsulation efficiency values of microcapsules with core to coating ratio of 1:10 and 1:20 and coating with different MD: Gum arabic ratios ; 10:0 ( ), 8:2 ( ) and 6:4 ( ) Different letters shows significant difference ($p \leq 0.05$)

The highest efficiency values were found when only maltodextrin was used as a coating material in the case of both 1:10 and 1:20 core to coating ratios (Figure 3.1). When 1:10 core to coating ratio was used, efficiency values of maltodextrin and gum arabic combination coating decreased with increasing gum arabic concentration. This could be explained by inefficient mixing due to the high viscosity of coating solution. The reverse condition was observed in capsules with core to coating ratio of 1:20. Gum arabic had emulsifying and stabilizing ability by help of its highly branched sugar

arrangements. Moreover, it could form strong protective matrix around the core material. This resulted in higher encapsulation efficiency values with increasing gum arabic concentration in coating material (Zuidam & Nedović, 2010).

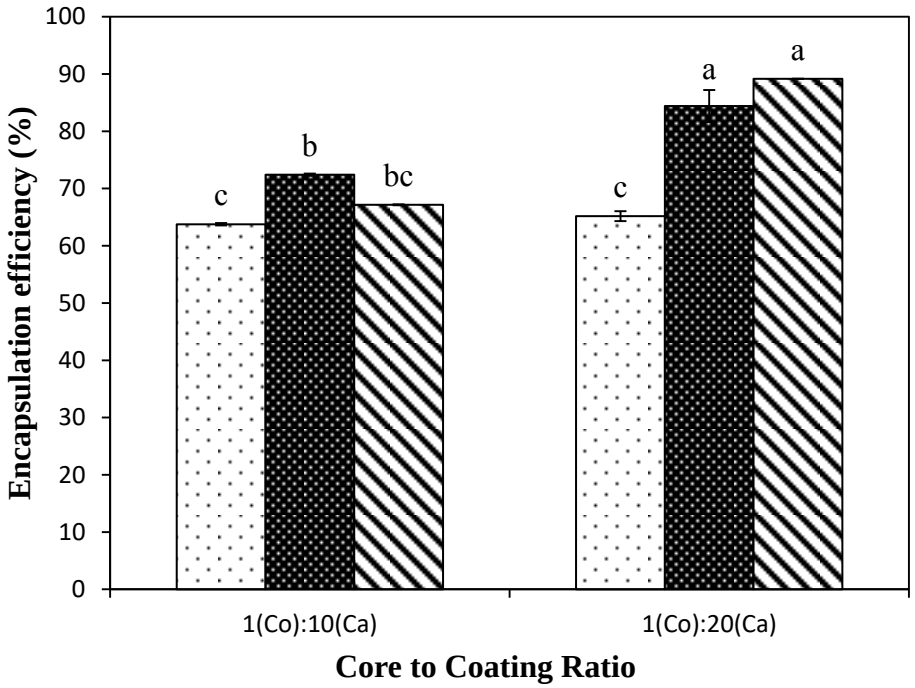


Figure 3.2 The encapsulation efficiency values of microcapsules with core to coating ratio of 1:10 and 1:20 and coating with different MD:Casein ratios ; 10:0 ( ), 8:2 ( ) and 6:4 ( ). Different letters shows significant difference ($p \leq 0.05$)

As can be seen in Figure 3.2, encapsulation efficiency values were smaller in microcapsules with core to coating ratio of 1:10. Even the small increase in casein concentration, could result in significant changes in solution viscosity (Konstance & Strange, 1991). This viscosity change had an impact on the homogenous emulsification process. As core to coating ratio increased, the homogeneity of microcapsules would decrease (Hogan et al., 2001). The homogeneity of casein containing coating was not a problematic issue for core to coating ratio of 1:20. However, the coalescence of capsules with core to coating ratio of 1:10 were negatively affected by the increase in casein concentration. This resulted in lower encapsulation efficiency. The highest encapsulation efficiency value was observed in maltodextrin-casein combination coating. This combination kept almost all the total phenolics inside the capsule. Thus, maltodextrin casein combination with 6:4 and 8:2 coating ratios and core to coating ratio of 1:20 provided the best microcapsules. Eventhough gums are commonly used as a encapsulating agent, they had poor interfacial properties and should have combined with other coating materials (Hogan et al., 2001). Due to protein molecule conformation, they have amphiphilic characteristics and high emulsification property. In particular, sodium caseinate allowed better coating around the core material and as a consequence higher stabilization of emulsion (Hogan et al., 2001; Madene et al., 2006).

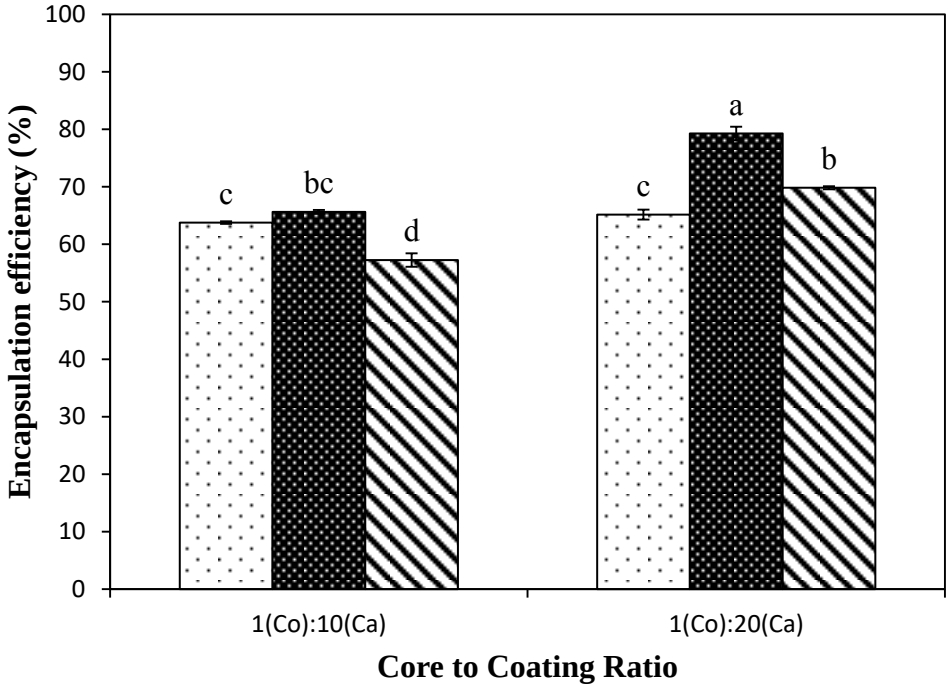


Figure 3.3 The encapsulation efficiency values of microcapsules with core to coating ratio of 1:10 and 1:20 and coating with different MD:WPC ratios; 10:0 ( ), 8:2 ( )and 6:4 ( ) Different letters shows significant difference ($p \leq 0.05$)

As can be seen in Figure 3.3, as maltodextrin: WPC ratios changed from 8:2 to 6:4, the encapsulation efficiency values decreased (Table B.6). The increasing proportion of maltodextrin gave higher bulk density and resulted in more compact physical structure when combined with WPC (Bae & Lee, 2008). However, the optimum proportion of maltodextrin and WPC should be determined according to core material. When maltodextrin:WPC ratio was 8:2, the optimum synergistic effect was obtained in terms of encapsulation efficiency. This led to more efficient encapsulation process.

As can be seen from Table 3.2, the lower encapsulation efficiency values were observed in only maltodextrin based coatings compared to maltodextrin-protein coating material combination. Though, carbohydrate based coatings are cost effective and has abundant sources, they are not usually used alone due to the lack of emulsifying property. The protein or lipid combination with carbohydrates can give higher protection of core material, and higher encapsulation efficiency values (Rosenberg & Sheu, 1995). Casein can keep phenolic compounds inside by help the of strong interaction between phenolic compounds and casein molecules (Helal, Desobry, Banon, & Shamsia, 2016). The same trend was also observed in maltodextrin and WPC combination coating. Proteins had both hydrophilic and hydrophobic groups and in encapsulation process, they could decrease the surface tension between core and coating material and contributed to the stability of the emulsion (Abascal & Gracia-Fadrique, 2009; Kitabatake & Doi, 2006)

3.2 Antioxidant activities of microcapsules

As can be seen in Table 3.3, the antioxidant activity of phenolic powder without coating had the largest value of 41.07 ppm DPPH˙/g dry weight. Since antioxidant materials are easily degradable with extrinsic factors like temperature changes, light and oxygen during encapsulation process, the antioxidant activity of the capsules were found to be lower than phenolic powder (Cordenunsi et al., 2005; Réblová, 2012). Since microcapsules were freeze dried twice, and exposed to light, their antioxidant activity decreased significantly. In literature, it was shown that the antioxidant properties of strawberry fruit was decreased after freeze drying process (Wojdylo, Figiel, & Oszmiański, 2009).

Total phenolic content and antioxidant activity results were correlated with correlation coefficient of 0.837 ($p=0.000$). The correlation between phenolic compounds and antioxidant activity were also observed by other researchers (Cordenunsi et al., 2005; Nile & Park, 2016; Somawathi, Rizliya, Wijesinghe, & Madhujith, 2014).

Table 3.3 Antioxidant activities of capsules having different coating type, coating ratio and core to coating ratio

Coating type	Coating ratio	Core: Coating ratio	Antioxidant activity(ppm DPPH˙/ g dry weight)
MD	10:0	1:20	$14.36\pm0.51^{bcd*}$
MD:GA	8:2	1:20	14.16 ± 0.44^{bcd}
MD:GA	6:4	1:20	13.70 ± 0.3^{bcd}
MD:Casein	8:2	1:20	11.03 ± 0.72^{d}
MD:Casein	6:4	1:20	11.10 ± 0.49^{d}
MD:WPC	8:2	1:20	13.48 ± 0.34^{cd}
MD:WPC	6:4	1:20	13.75 ± 0.25^{bcd}
MD	10:0	1:10	23.86 ± 0.89^{a}
MD:GA	8:2	1:10	24.20 ± 1.25^{a}
MD:GA	6:4	1:10	26.05 ± 1.16^{a}
MD:Casein	8:2	1:10	20.63 ± 1.15^{abc}
MD:Casein	6:4	1:10	19.61 ± 0.59^{abcd}
MD:WPC	8:2	1:10	21.50 ± 1.22^{abc}
MD:WPC	6:4	1:10	22.30 ± 0.62^{ab}
Phenolic powder			41.07 ± 1.83

*Different letters within the same column shows significant difference ($p \leq 0.05$)

Core to coating ratio and coating material type were found to have significant effect ($p \leq 0.05$) on antioxidant activity (Table B.7). As expected, microcapsules core to coating ratio of 1:10 had higher antioxidant activity than the ones prepared with core to coating ratio of 1:20. This is due to the fact that the capsules with core to coating ratios of 1:20 and 1:10, contained 1 g and 2 g of the concentrated phenolic powder, respectively.

Figure 3.4- Figure 3.6 showed the effect of the coating material type and core to coating ratio on antioxidant activity. No significant difference was determined between combination of different coating material types. According to 2 way ANOVA results (Table B.8-Table B.10), antioxidant activity of microcapsules which were coated with only maltodextrin and maltodextrin-gum arabic combination were not statistically different from microcapsules having maltodextrin-casein and maltodextrin-WPC combination coating material (Table 3.3).

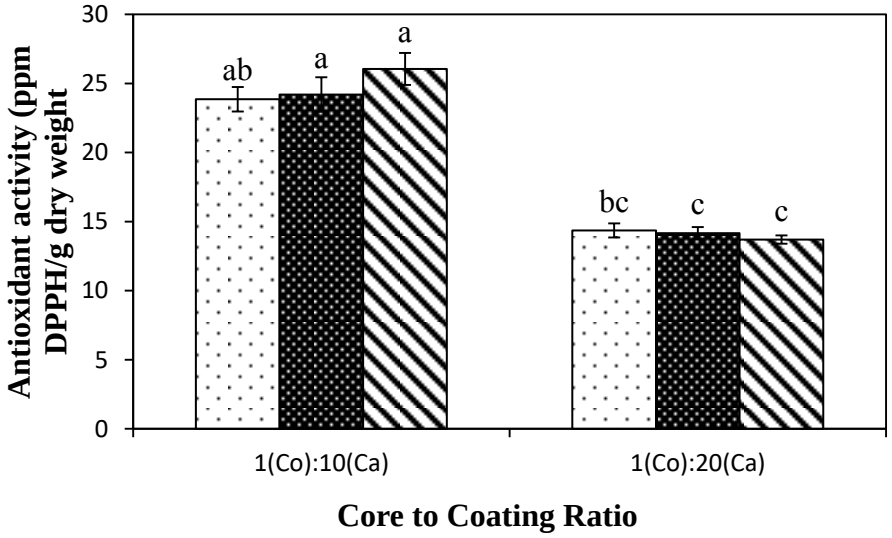


Figure 3.4 The antioxidant activity values of microcapsules with core to coating ratio of 1:10 and 1:20 and coating with different MD: Gum arabic ratios ; 10:0 ( ), 8:2 ( ) and 6:4 ( ). Different letters shows significant difference ($p \leq 0.05$)

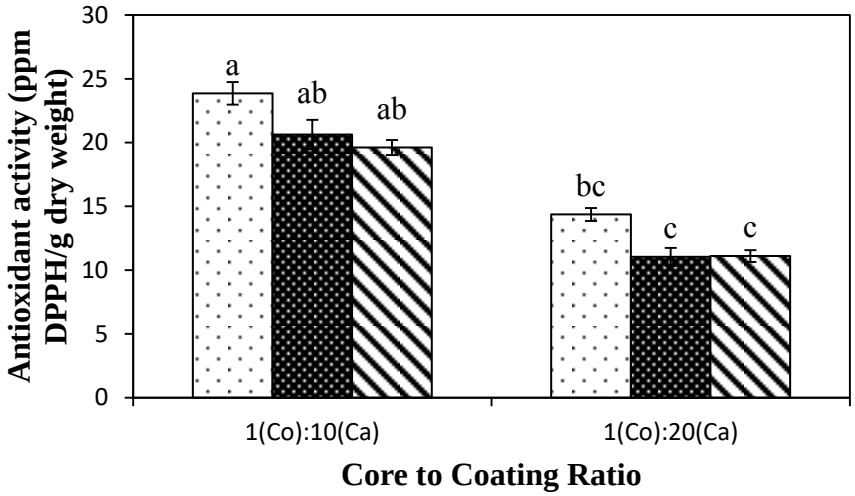


Figure 3.5 The antioxidant activity values of microcapsules with core to coating ratio of 1:10 and 1:20 and coating with different MD: Casein ratios ; 10:0 ( ), 8:2 ( ) and 6:4 ( ). Different letters shows significant difference ($p \leq 0.05$)

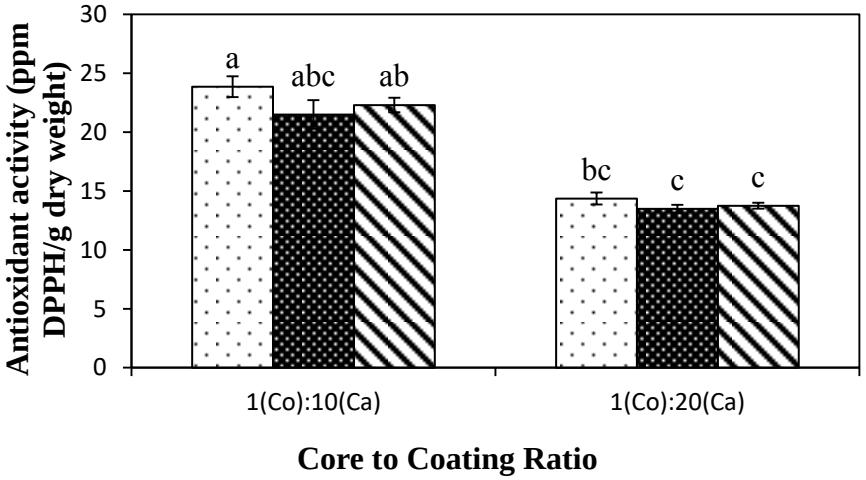


Figure 3.6 The antioxidant activity values of microcapsules with core to coating ratio of 1:10 and 1:20 and coating with different MD: WPC ratios ; 10:0 ( ), 8:2 ( ) and 6:4 ( ). Different letters shows significant difference ($p \leq 0.05$)

3.3 Particle size analysis

Particle size analysis of all emulsions were performed before freeze drying process. In Table 3.4, the particle size distribution was evaluated in terms of Sauter mean diameter (D32) values, the specific surface area (SSA(m^2/g**)**) and span values.

Table 3.4 Particle size analyses of microcapsules prepared with maltodextrin in combination with gum arabic, casein or WPC at different ratios (10:0, 8:2, 6:4) and with different core to coating ratios (CCR) (1:20, 1:10)

Coating type	Coat ratio	CCR	D32 (µm)	Span	Specific surface area(m^2/g)
MD	10:0	1:20	$6.80\pm0.21^{e*}$	$2.40\pm0.02^{gh*}$	$0.92\pm0.009^{f*}$
MD:GA	8:2	1:20	7.61 ± 0.01^{de}	4.40 ± 0.17^{de}	0.79 ± 0.001^{gh}
MD:GA	6:4	1:20	8.71 ± 0.15^{c}	3.47 ± 0.01^{fg}	0.81 ± 0.006^{g}
öMD:Csn	8:2	1:20	2.44 ± 0.06^{hı}	8.77 ± 0.08^{b}	2.48 ± 0.019^{b}
MD:Csn	6:4	1:20	5.38 ± 0.34^{f}	6.51 ± 0.08^{c}	1.18 ± 0.006^{e}
MD:WPC	8:2	1:20	3.40 ± 0.01^{g}	8.93 ± 0.06^{b}	1.77 ± 0.003^{d}
MD:WPC	6:4	1:20	1.67 ± 0.02^{ı}	11.27 ± 0.03^{a}	3.63 ± 0.009^{a}
MD	10:0	1:10	14.15 ± 0.05^{a}	1.52 ± 0.05^{h}	0.42 ± 0.002^{ı}
MD:GA	8:2	1:10	8.45 ± 0.13^{cd}	3.76 ± 0.05^{ef}	0.71 ± 0.010^{h}
MD:GA	6:4	1:10	13.2 ± 0.20^{b}	3.09 ± 0.39^{fg}	0.46 ± 0.007^{ı}
MD:Csn	8:2	1:10	5.43 ± 0.16^{f}	4.82 ± 0.05^{d}	1.11 ± 0.033^{e}
MD:Csn	6:4	1:10	7.44 ± 0.26^{e}	5.19 ± 0.09^{d}	0.81 ± 0.028^{g}
MD:WPC	8:2	1:10	5.23 ± 0.15^{f}	4.95 ± 0.01^{d}	1.15 ± 0.033^{e}
MD:WPC	6:4	1:10	3.03 ± 0.03^{gh}	7.17 ± 0.11^{c}	1.98 ± 0.015^{c}
Phenolic powder			1.64 ± 0.19	18.05 ± 1.64	3.68 ± 0.40

*Different letters within the same column shows significant difference at $p \leq 0.05$

As it can be seen from Table 3.4, core to coating ratio had a significant effect on particle size distribution values (Table B.11-Table B.13). Capsules with 1:20 core to coating ratio had smaller Sauter mean diameter (D32) value than those with 1:10 core to coating ratio. For core to coating ratio of 1:20, Sauter mean diameter ranged between 1.67 μm and 8.71 μm. The average particle size of samples of 1:10 core to coating ratio capsules varied between 3.03 μm and 14.15 μm. In order to get capsules, high speed homogenizer was used. In literature, the droplet size of the capsules with high speed homogenizer ranged from 2 to 10 μm in diameter which was similar to the findings of this study (McClements, 2005). Figure 3.7 - Figure 3.9 showed particle size distribution of the capsules with different coating materials. It was clearly seen that as core to coating ratio changed from 1:20 to 1:10, the particle size distribution curve shifted to larger particle size side (Figure 3.7- 3.9). In other words, as phenolic powder concentration increased in emulsion, the particle size would be larger and coalescence of the emulsion would increase. Hogan et al., (2001) reported that average particle size value increased with increasing core material. In order to get more stable emulsions, smaller capsules were more preferred than larger ones. Since core material concentration increased, the coating material was insufficient to encapsulate it and this resulted in coalescence of particles and larger droplet size when core to coating ratio was 1:10.

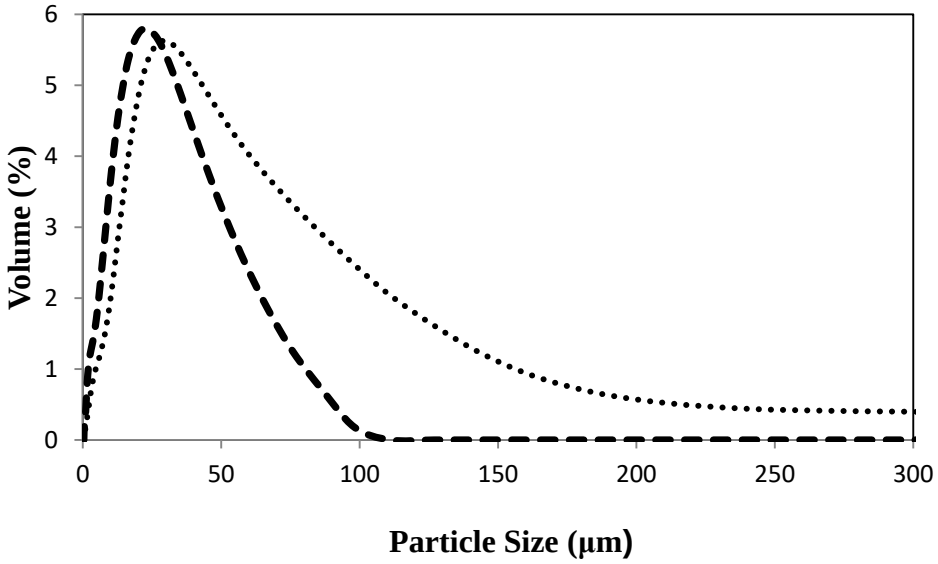


Figure 3.7 Particle size distributions of microcapsules prepared with MD: GA at a ratio of 6:4 and with different core to coating ratios; 1:10 (dotted line) and 1:20 (dashed line)

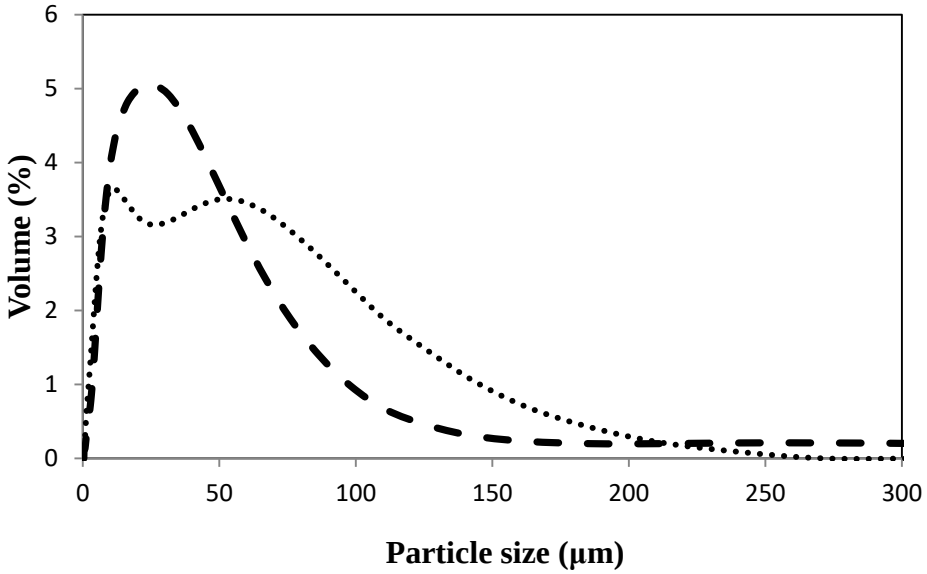


Figure 3.8 Particle size distributions of microcapsules prepared with MD: Casein at a ratio of 6:4 with different core to coating ratios; 1:10 (dotted line) and 1:20 (dashed line)

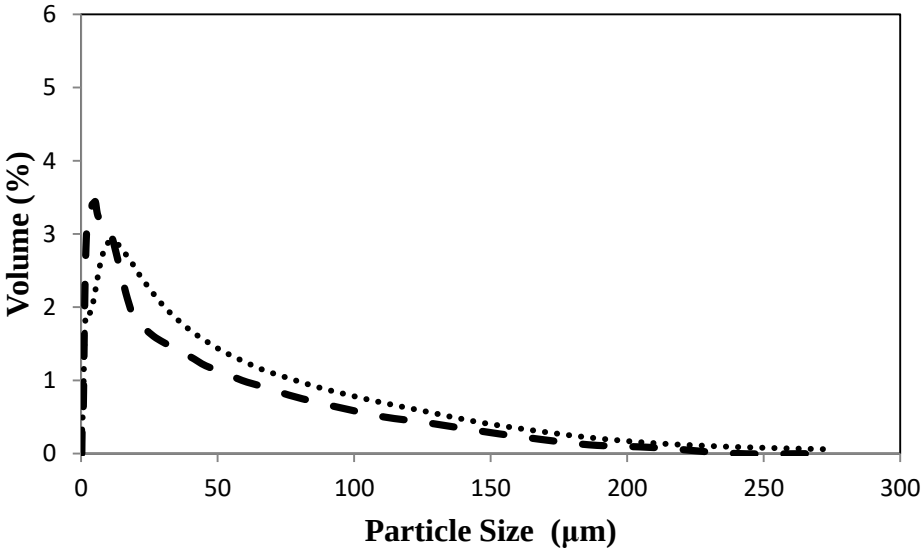


Figure 3.9 Particle size distributions of microcapsules prepared with MD: WPC at a ratio of 6:4 with different core to coating ratios; 1:10 (dotted line) and 1:20 (dashed line)

There was an inverse relationship between D32 value and span values. In addition, D32 value and specific surface area were inversely correlated. Sauter mean diameter value increased with core material concentration, while specific surface area and span values of capsules with core to coating ratio of 1:10 were lower than capsules with core to coating of 1:20. The increasing particle size resulted in a decrease in specific surface area value. Similar result was also obtained by Cilek et al., (2012).

Type of coating material affected the particle size distribution significantly (Table 3.4). In general, coatings containing only carbohydrate based materials had larger D32 values as compared to coating materials with carbohydrate and protein combination. The protein based materials are known to change the surface tension of the emulsion. This gives the particle

size distribution differences between different coating materials (Rosenberg & Sheu, 1995). It was previously shown that the carbohydrate based coating materials led to coarser emulsion in terms of particle size distribution and poorer retention of core material as compared to protein based coating materials (Baranauskiene et al., 2006).

As can be seen from Figure 3.10, higher gum arabic concentration resulted in higher particle size value. Moreover, microcapsules with core to coating ratio of 1:10 had the highest sauter mean diameter. The statistical differences was shown in Table B.14.

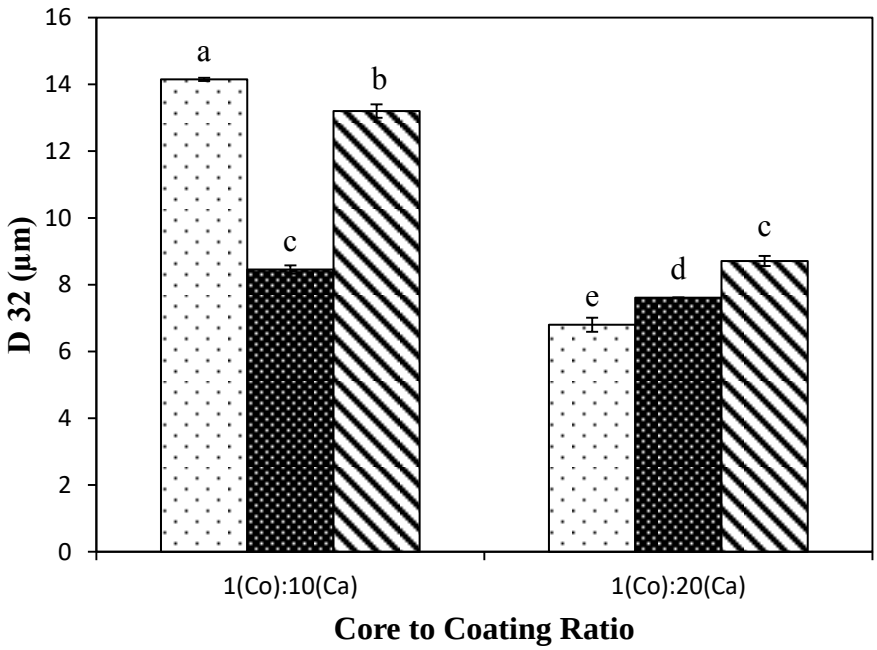


Figure 3.10 Sauter mean diameter of microcapsules with core to coating ratio of 1:10 and 1:20 and coating with different MD:Gum Arabic ratios; 10:0 ( ), 8:2 ( ) and 6:4 ( ). Different letters shows significant difference ($p \leq 0.05$)

As can be seen from Figure 3.11, microcapsules with only maltodextrin coating had the higher D32 value than microcapsules with maltodextrin-casein combination coating. In capsules with maltodextrin-casein combination, the sauter mean diameter increased with casein concentration. Konstance & Strange (1991) showed that the casein concentration had a significant effect on the viscosity of emulsion and this resulted in larger D32.

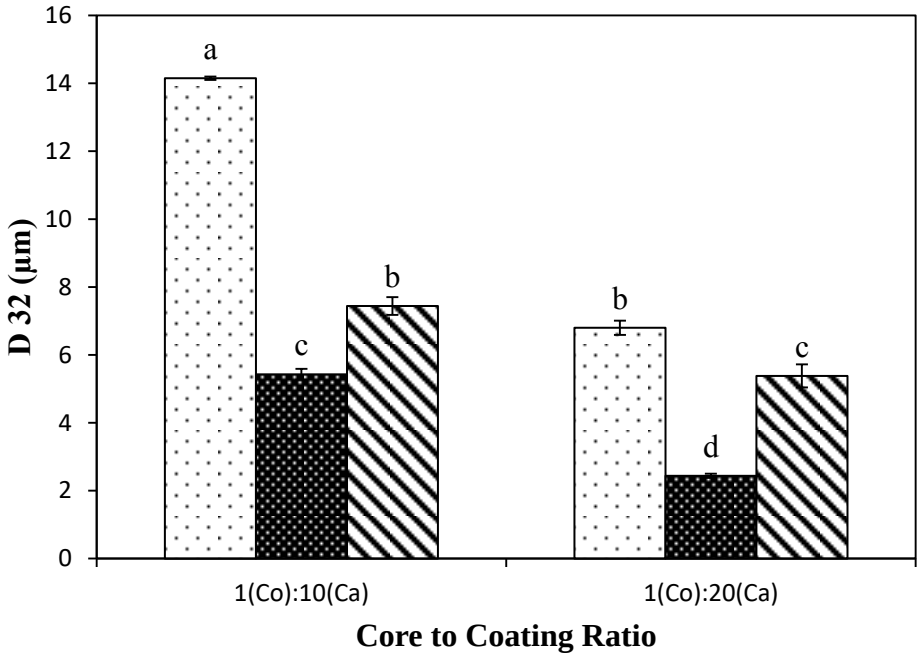


Figure 3.11 Sauter mean diameter of of microcapsules with core to coating ratio of 1:10 and 1:20 and coating with different MD:Casein ratios; 10:0 ( ), 8:2 ( ) and 6:4 ( ). Different letters shows significant difference ($p \leq 0.05$)

Figure 3.12, showed that the highest value of Sauter mean diameter was obtained in only maltodextrin coating in both core to coating ratios (Table B.16). The ones with higher concentration of WPC had smaller particles. Rosenberg & Sheu (1995) investigated the effect of coating material ratio on ethyl caprylate encapsulation with maltodextrin-WPC coating. They found that as WPC: maltodextrin ratio changed from 1:19 to 1:9, D32 value of microcapsules decreased due to the surface tension decreasing ability of WPC. Moreover, as a coating material, WPC contributed to film-forming and emulsifying ability and maltodextrin acted as matrix forming agent (Rosenberg & Sheu, 1995).

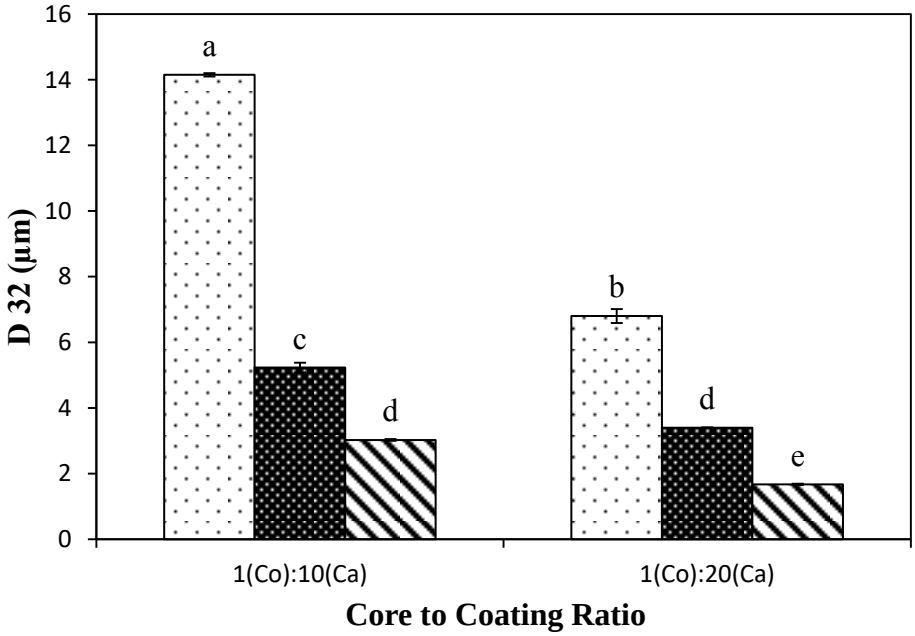


Figure 3.12 Sauter mean diameter of microcapsules with core to coating ratio of 1:10 and 1:20 and coating with different MD:WPC ratios; 10:0 ( ), 8:2 ( ) and 6:4 ( ). Different letters shows significant difference ($p \leq 0.05$)

3.4 Morphological analysis of microcapsules

The microscopic images were obtained for maltodextrin, maltodextrin:gum arabic, maltodextrin:casein and maltodextrin:WPC coated microcapsules in order to show the differences between carbohydrate and carbohydrate-protein combination as a coating material. The ratios were 10:0 and 8:2 respectively.

In Figure 3.13, it was clearly seen that for all coating material types, microcapsules having 1:10 core to coating ratio were bigger in size as compared with 1:20 ratios. This conclusion was consistent with the particle size analysis results (Table 3.4). Since the concentration of phenolic powder without coating was high in microcapsules with 1:10 core to coating ratio, the amount of coating material was insufficient for encapsulation and this resulted in coalescence of particles.

Moreover, from the microscopic images, maltodextrin-WPC combination resulted in smaller particles than only maltodextrin and maltodextrin-gum Arabic coated ones. As explained in Section 3.3, protein addition to coating material changed the size of the microcapsules and this lead to different outlook of microcapsules due to protein's ability of changing surface tension of emulsion (Baranauskiene et al., 2006). This conclusion was clearly seen in Figure 3.13.

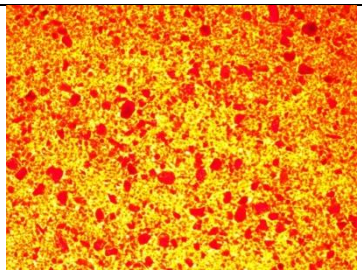
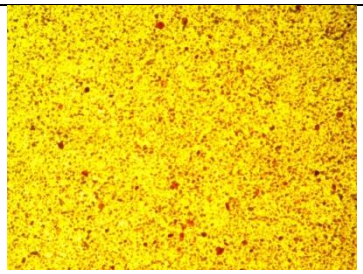
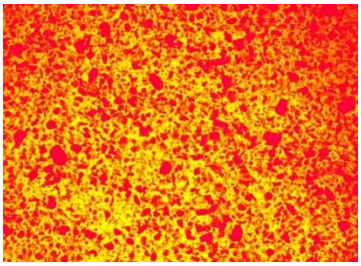
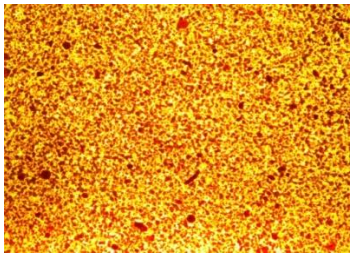
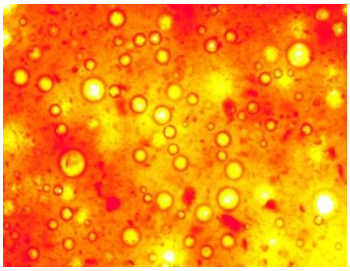
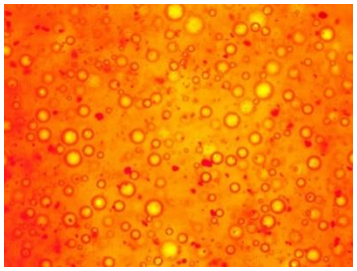
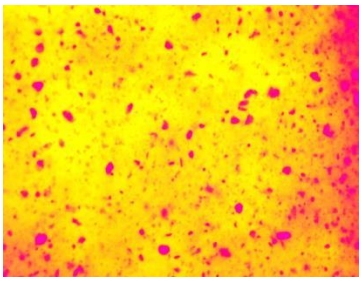
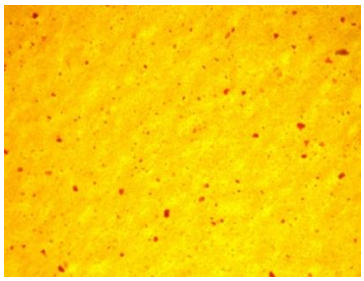
	1:10 core to coating ratio	1:20 core to coating ratio
MD (10:0)		
MD: Gum Arabic (8:2)		
MD: Casein (8:2)		
MD: WPC (8:2)		

Figure 3.13 Optical images of microcapsules having 1:10 and 1:20 core to coating ratios

3.5 Heat stability of microcapsules

Natural phenolic compounds are highly susceptible to light, heat and oxygen. They can be easily destroyed during storage (Cilek et al., 2012). Encapsulation provides an effective barrier protecting the core material. Emulsification of core and coating material is critical to get stable capsules.

In this study, the stability of capsules exposed to heat treatment was evaluated. Heat stability was measured for capsules with core to coating ratio of 1:20 because of their higher encapsulation efficiency values. Heat stability of microcapsules with different coating material types were evaluated in terms of total phenolic compounds at 80°C. In addition, phenolic powder without coating was tested against thermal degradation of phenolic compounds.

Variation of total phenolic content of microcapsules prepared with maltodextrin:casein and maltodextrin:WPC at different ratios and also phenolic powder without coating, with respect to heating time were shown in Figure 3.14 -Figure 3.16 and statistical differences between coating ratios of 6:4 and 8:2 were evaluated (Table B.17 and Table B.18).

As can be seen from the Figure 3.14 phenolic content of phenolic powder without coating showed a significant decrease in 1 hour at 80°C (Table B.19). The total phenolic loss was 45.88% after 1 hour heat treatment. This decrease was 1.79%-3.92% and 33.58%-9.46% in casein and whey protein containing capsules having 6:4 and 8:2 ratios, respectively. Statistical analysis showed that there was not considerable loss in total phenolic content of microcapsules during 1 hour in contrast to that phenolic powder without coating. This showed that capsules were efficient in terms of heat stability.

In phenolic powder, total phenolic content first increased and then decreased after 1 hour. The same kind of fluctuation was observed in various studies which investigated the total phenolic content change of grape seed flour, apple pomace and six onion varieties during different time and temperature combinations (Candrawinata, Golding, Roach, & Stathopoulos, 2014; Ross, Hoye, & Fernandez-Plotka, 2011; Sharma et al., 2015). Phenolic compounds can be classified in different classes as mentioned in Section 1.1. By heat application some types may increase, while certain types can decrease. Thus, the generalization of the alteration of total phenolic content by thermal treatment was not possible. The increase in total phenolic content during heating was related to the cleavage of esterified and glycosylated bond of phenolic compounds. Thus, the bonded phenolics were free and this resulted in increase in total phenolic content (Sharma et al., 2015). In addition, the interconversion of phenolic compounds between themselves was another reason for increasing total phenolic content (Soong & Barlow, 2004). On the other hand, since they were sensitive compounds against external factors, the continuous heating could degrade the phenolic compounds after a certain point (Cavalcanti et al., 2011). The degradation time and temperature for phenolics can vary for different types.

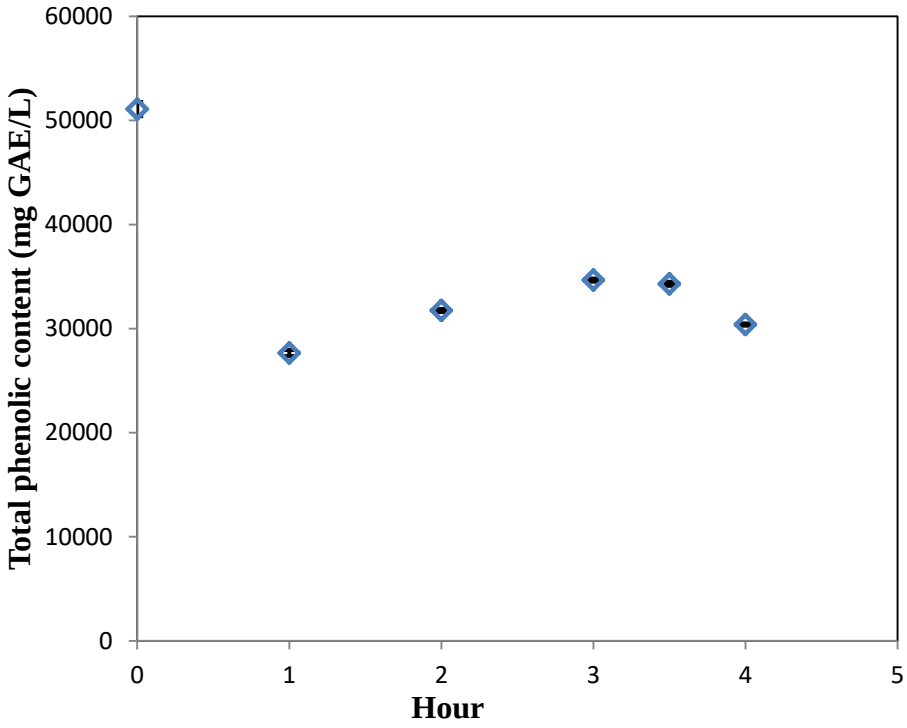


Figure 3.14 Variation of total phenolic content values of phenolic powder without coating with respect to time when they are kept at 80°C.

As mentioned in section 3.1 total phenolic content of the phenolic compounds extracted from onion skin was lost during encapsulation procedure containing homogenization and freeze drying steps. Mechanical forces damaged phenolic compounds. Thus, in the heat stability graphs, the starting point of the total phenolic content of capsules and phenolic powder without coating cannot be same. The behaviour of total phenolic content of microcapsules was highly correlated with the duration of heat treatment. As time increased, the total phenolic content of capsules decreased.

Experimental results showed that after 4 hour heat treatment, microcapsules coated with combination of maltodextrin: casein lost their overall phenolic compounds by 40.63% and 15.39% for 6:4 and 8:2 ratios, respectively (Figure 3.15). In addition, phenolic content losses after 4 hour heat application were 42.83% and 26.88% for microcapsules prepared with maltodextrin and WPC at 6:4 and 8:2 ratios, respectively (Figure 3.16). This showed that maltodextrin-casein coating was more efficient than maltodextrin-WPC coating material in preservation of phenolic compounds. Since heating of whey protein above 70°C resulted in protein unfolding, especially β-lactoglobulin changed its conformation. Above a certain level of temperature, unfolded protein led to coagulation of protein molecule (Chevallier et al., 2016). Among milk proteins, whey was the most susceptible protein to denaturation (Mcgrath, Kinsella, Huppertz, Mcsweeney, & Kelly, 2016). WPC containing coating material broke down and could not protect the core material anymore after a certain temperature.

On the other hand, casein especially in sodium caseinate form was known to be very heat stable. The heat stability of casein protein was due to lacking of the secondary and tertiary structure (Broyard & Gaucheron, 2015; Mcgrath et al., 2016). As a result, loss of total phenolic content was less in the case of maltodextrin-casein combination coating material.

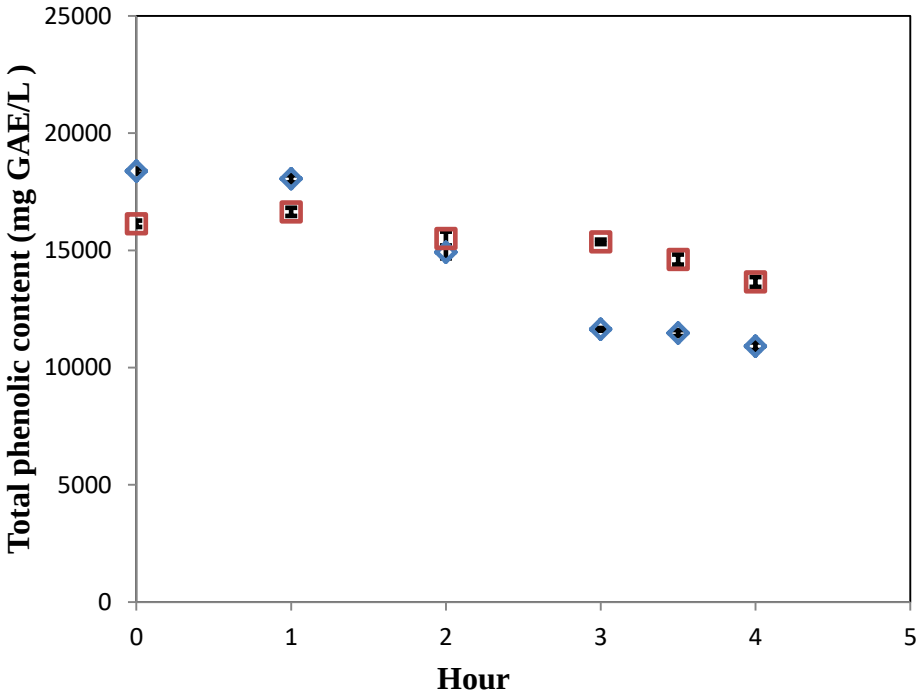


Figure 3.15 Variation of total phenolic content values of maltodextrin: casein microcapsules with 6:4 (◇) and 8:2 (□) ratios with respect to time when they are kept at 80°C.

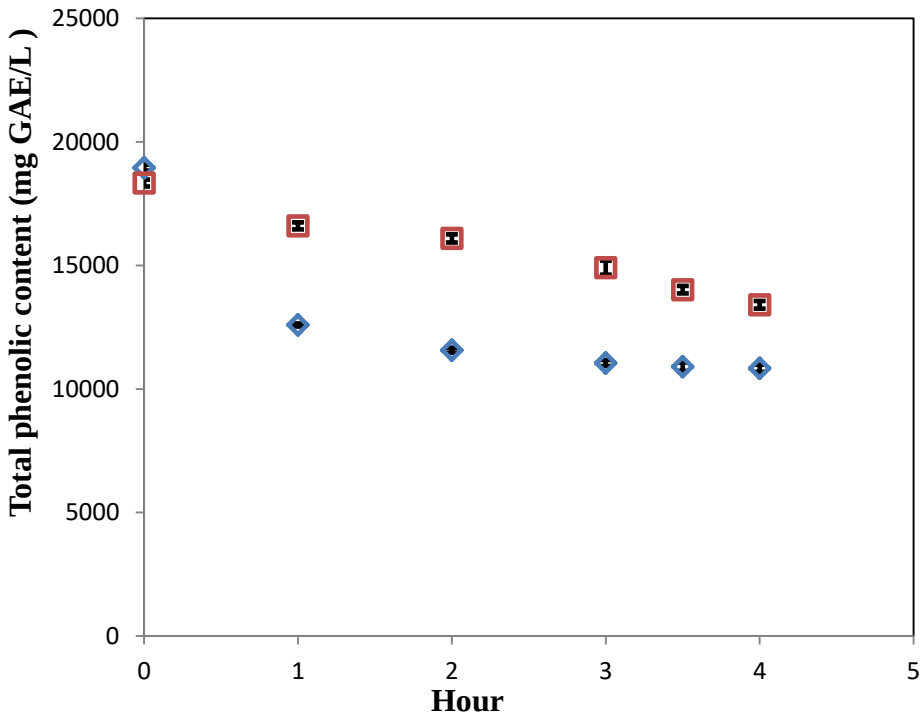


Figure 3.16 Variation of total phenolic content values of maltodextrin: WPC microcapsules with 6:4 (◇) and 8:2 (□) ratios with respect to time when they are kept at 80°C.

Table 3.5 Antioxidant activity of microcapsules prepared with maltodextrin:casein and maltodextrin:WPC at 8:2 ratio and with 1:20 core to coating ratio before heat treatment and after 4 hour heat treatment

Coating type	**Initial Antioxidant activity (mg DPPH˙/L)**	**Antioxidant activity after 4 hour (mg DPPH˙/L)**
MD-Casein(8:2)	1103.41±71.47	1952.55±89.56
MD-WPC (8:2)	1348.24±34.06	1729.93±123.84
Phenolic powder without coating	4106.75±182.60	3656.93±104.07

The stability of capsules was also evaluated in terms of retention of antioxidant activity after heating for 4 hours. Antioxidant activity is mostly related to the phenolic compounds (Lou, Lin, Hsu, Chiu, & Ho, 2014). As it can be seen from Table 3.5, phenolic powder without coating lost antioxidant activity during heating, on the other hand, the antioxidant activity of microcapsules increased. Phenolic powder without coating was more prone to external factors than encapsulated phenolic powder. Therefore, the thermal decomposition of phenolic compounds resulted in loss of antioxidant activity (Luca, Cilek, Hasirci, Sahin, & Sumnu, 2014; Songsungkan & Chanthai, 2014). On the other hand, in microcapsules the antioxidant activity values ascended after 4 hour heat treatment (Table 3.5). As mentioned in Section 1.2, onion skin contained phenolic compounds,

dietary fibre and nonstructural carbohydrate molecules such as fructose, glucose and sucrose (Benítez et al., 2011). Sugar molecules and proteins created Maillard browning reaction at high temperature values. Reducing sugar molecule reacted with the free amino group of protein molecules and after some continuous stages, they gave condensation product of brown pigments called melanoidin (Martins, Jongen, & Boekel, 2001). Color change was observed in microcapsules after heating. These Maillard compounds had antioxidant activity (Castillo, Villamiel, Moreno, & Corzo-Martínez, 2006; Nicoli, Anese, Parpinel, Silvia, & Lericia, 1997). Thus, the overall antioxidant activity increased in microcapsules. Another reason of the increasing amount of antioxidant activity of microcapsules was the adverse relation of antioxidant activity with different kinds of phenolic compounds by heat treatment. Phenolic compounds had various classes like flavonoids, phenolic acids or lignins (Vermerris & Nicholson, 2008). The measurement was done in terms of total phenolic compounds. Eventhough the amount of total phenolics decreased, the content of the individual phenolics might increase. In other words, by heat treatment, flavonoids increased, while phenolic acids decreased or vice versa. The antioxidant content of the microcapsules were strongly related to the active ingredient of the total phenolics (Settharaksa, Jongjareonrak, Hmadhlu, Chansuwan, & Siripongvutikorn, 2012). Active phenolic compound could change with respect to the degree of heat treatment. Therefore, the behaviour of antioxidant activity was different from the behaviour of total phenolic content after 4 hour.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In this study, in order to determine the best encapsulation formulation, onion skin phenolic powder was coated with different coating materials at different combinations and different core to coating ratios. The encapsulation efficiency, total phenolic content, antioxidant activity, particle size distribution, heat stability and morphology of microcapsules were investigated.

Among two different core to coating ratios, 1:20 ratio provided the highest encapsulation efficiency. In the particle size analysis of microcapsules, as core to coating ratio changed from 1:20 to 1:10, capsules with coarser size were obtained. The sauter mean diameter and the specific surface area values were inversely proportional with each other. Moreover, as core to coating ratio was changed from 1:20 to 1:10 span values decreased. Addition of protein to coating reduced particle size of capsules significantly. By changing the coating material combination ratios, significantly different particle size distribution could be obtained. On the other hand; no significant difference between different coating types in terms of antioxidant activity.

Addition of casein to coating increased the encapsulation efficiency and heat stability of microcapsules. Thus, coating with maltodextrin-casein combination can be recommended to be used in encapsulation of phenolic compounds.

For the future study, encapsulated phenolic powder can be added to a food material in order to utilise high amount of phenolic compounds of onion skin. The bioavailability of phenolic compounds of onion skin can also be investigated. Another recommendation can be to use different kind of drying method such as spray drying for the microcapsules.

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APPENDICES

APPENDIX A

CALIBRATION CURVES

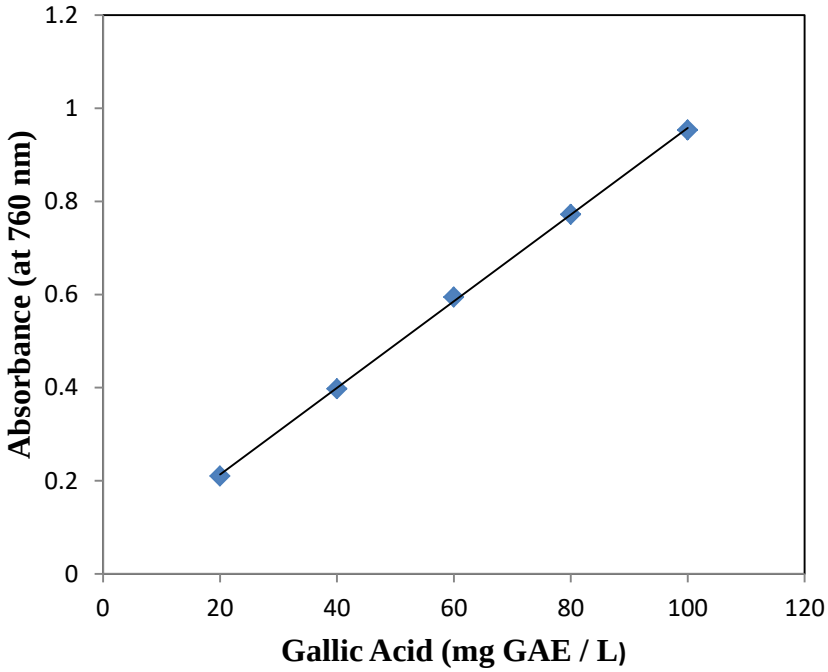

Figure A.1 Calibration curve prepared by gallic acid in ethanol:acetic acid:water mixture (50:8:42 (v/v)) for determination of total phenolic contents.

Absorbance (760 nm) = 0.0093 * (mg GAE / L) + 0.0274

$r^2 = 0.9997$

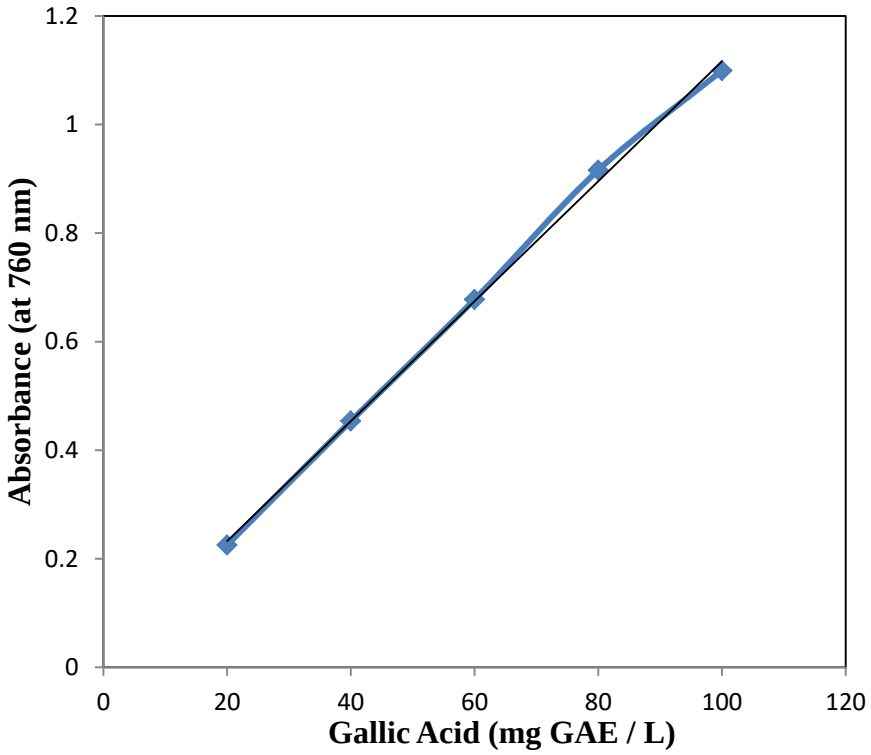

Figure A.2 Calibration curve prepared by gallic acid in ethanol:water mixture (50:50 (v/v)) for determination of total phenolic contents.

Absorbance (760 nm) = 0.0111 * (mg GAE / L) + 0.0113

$r^2 = 0.9984$

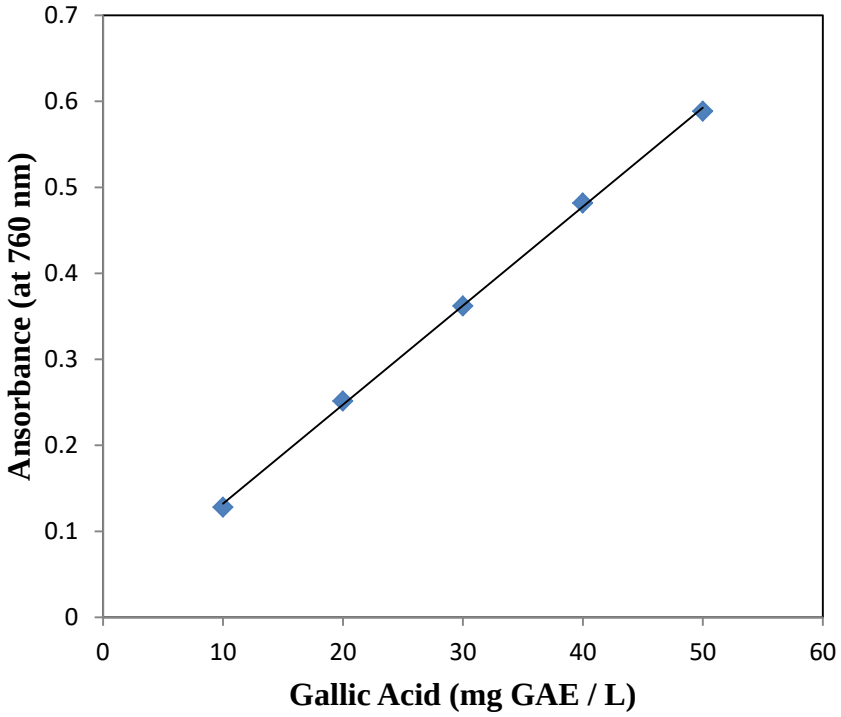

Figure A.3 Calibration curve prepared by gallic acid in ethanol:methanol mixture (50:50 (v/v)) for determination of surface phenolic contents

Absorbance (760 nm) = 0.0115 * (mg GAE / L) + 0.017

$r^2 = 0.9995$

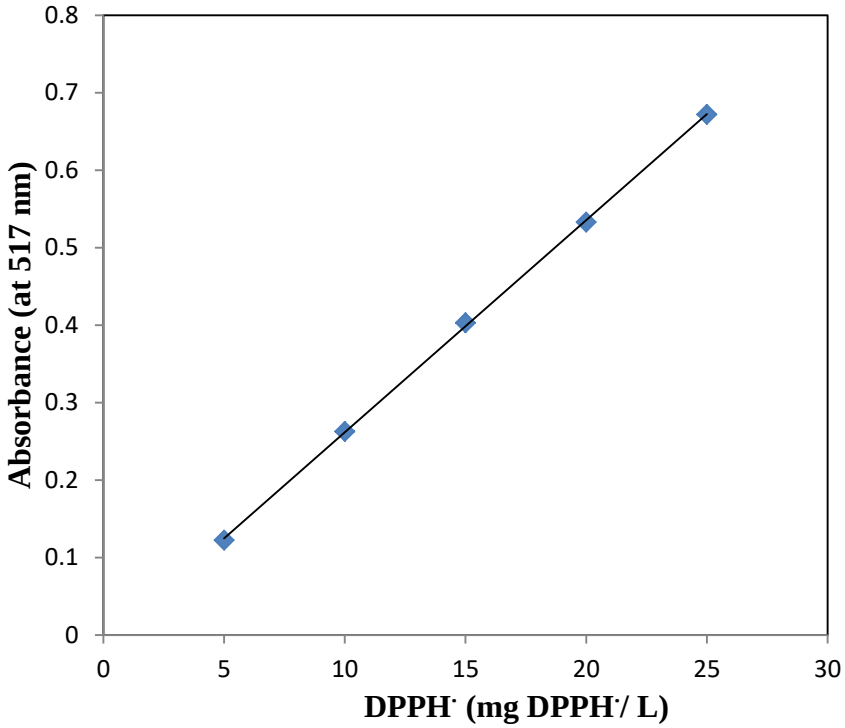

Figure A.4 Calibration curve prepared by DPPH· in methanol for determination of antioxidant activity.

Absorbance (517nm) = 0.0274 * (mg GAE / L) - 0.0123

$r^2 = 0.9998$

APPENDIX B

STATISTICAL ANALYSES

Table B.1 Two way ANOVA and Tukey's comparison test for total phenolic content

Factor	Type	Levels	Values
ratıo	fixed	7	1; 2; 3; 4; 5; 6; 7
core coat	fixed	2	1:10; 1:20

Analysis of Variance for TPC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
ratıo	6	19463,9	19463,9	3244,0	537,04	0,000
core coat	1	43529,8	43529,8	43529,8	7206,38	0,000
ratıo*core coat	6	5256,0	5256,0	876,0	145,02	0,000
Error	14	84,6	84,6	6,0		
Total	27	68334,3				

S = 2,45773 R-Sq = 99,88% R-Sq(adj) = 99,76%

Table B.1 (Continued)

Unusual Observations for TPC

Obs	TPC	Fit	SE Fit	Residual	St Resid
21	179,742	183,344	1,738	-3,602	-2,07 R
28	186,946	183,344	1,738	3,602	2,07 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

ratıo	N	Mean	Grouping
2	4	278,5	A
3	4	276,6	A
1	4	268,8	B
7	4	232,9	C
6	4	225,7	D
4	4	222,0	D
5	4	211,5	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

core coat	N	Mean	Grouping
1:10	14	284,6	A
1:20	14	205,7	B

Means that do not share a letter are significantly different.

Table B.1 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

ratıo	core coat	N	Mean	Grouping
1	1:10	2	328,5	A
3	1:10	2	300,3	B
2	1:10	2	297,3	B
7	1:10	2	282,5	C
6	1:10	2	261,8	D
5	1:10	2	261,5	D
4	1:10	2	260,2	D
2	1:20	2	259,7	D
3	1:20	2	253,0	D
1	1:20	2	209,2	E
6	1:20	2	189,5	F
4	1:20	2	183,8	F
7	1:20	2	183,3	F
5	1:20	2	161,4	G

Means that do not share a letter are significantly different.

Table B.2 Two way ANOVA and Tukey's comparison test for surface phenolic content

Factor	Type	Levels	Values
ratıo	fixed	7	1; 2; 3; 4; 5; 6; 7
core coat	fixed	2	1:10; 1:20

Table B.2 (Continued)

Analysis of Variance for SPC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
ratıo	6	21196,9	21196,9	3532,8	497,35	0,000
core coat	1	14637,6	14637,6	14637,6	2060,68	0,000
ratıo*core coat	6	1924,9	1924,9	320,8	45,16	0,000
Error	14	99,4	99,4	7,1		
Total	27	37858,9				

S = 2,66520 R-Sq = 99,74% R-Sq(adj) = 99,49%

Unusual Observations for SPC

Obs	SPC	Fit	SE Fit	Residual	St Resid
19	29,887	25,211	1,885	4,676	2,48 R
26	20,536	25,211	1,885	-4,675	-2,48 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

ratıo	N	Mean	Grouping
2	4	122,9	A
3	4	117,9	A
1	4	96,0	B
6	4	84,5	C
7	4	67,6	D
4	4	52,7	E
5	4	48,7	E

Means that do not share a letter are significantly different.

Table B.2 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

core
coat N Mean Grouping
1:10 14 107,2 A
1:20 14 61,4 B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

core
ratıo coat N Mean Grouping
2 1:10 2 141,0 A
3 1:10 2 123,6 B
1 1:10 2 119,0 B C
3 1:20 2 112,2 C D
6 1:10 2 111,9 C D
2 1:20 2 104,7 D E
7 1:10 2 97,1 E
4 1:10 2 85,4 F
1 1:20 2 72,9 G
5 1:10 2 72,1 G
6 1:20 2 57,2 H
7 1:20 2 38,0 I
5 1:20 2 25,2 J
4 1:20 2 20,0 J

Means that do not share a letter are significantly different.

Table B.3 Two way ANOVA and Tukey's comparison test for encapsulation efficiency

Factor	Type	Levels	Values
ratıo	fixed	7	1; 2; 3; 4; 5; 6; 7
core coat	fixed	2	1:10; 1:20

Analysis of Variance for eff, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
ratıo	6	2063,20	2063,20	343,87	185,21	0,000
core coat	1	613,62	613,62	613,62	330,50	0,000
ratıo*core coat	6	421,28	421,28	70,21	37,82	0,000
Error	14	25,99	25,99	1,86		
Total	27	3124,09				

S = 1,36259 R-Sq = 99,17% R-Sq(adj) = 98,40%

Unusual Observations for eff

Obs	eff	Fit	SE Fit	Residual	St Resid
19	81,5750	84,3905	0,9635	-2,8155	-2,92 R
26	87,2060	84,3905	0,9635	2,8155	2,92 R

R denotes an observation with a large standardized residual.

Table B.3 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

ratıo	N	Mean	Grouping
5	4	78,4	A
4	4	78,2	A
7	4	72,5	B
1	4	64,5	C
6	4	63,5	C
3	4	57,2	D
2	4	56,1	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

core coat	N	Mean	Grouping
1:20	14	71,9	A
1:10	14	62,5	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

ratıo	core coat	N	Mean	Grouping
4	1:20	2	89,1	A
5	1:20	2	84,4	A B
7	1:20	2	79,3	B
5	1:10	2	72,4	C
6	1:20	2	69,8	C D
4	1:10	2	67,2	C D E
7	1:10	2	65,6	D E
1	1:20	2	65,2	D E

Table B.3 (Continued)

1	1:10	2	63,8	E F
2	1:20	2	59,7	F G
3	1:10	2	58,9	F G
6	1:10	2	57,3	G H
3	1:20	2	55,6	G H
2	1:10	2	52,6	H

Means that do not share a letter are significantly different.

Table B.4 Two way ANOVA and Tukey's comparison test for encapsulation efficiency of maltodextrin-gum arabic combination with different ratios

Factor	Type	Levels	Values
arab ratıo	fixed	3	6:04; 8:02; 10:00
coat ratıo	fixed	2	1:10; 1:20

Analysis of Variance for eff, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
arab ratıo	2	163,769	163,769	81,885	117,02	0,000
coat ratıo	1	9,496	9,496	9,496	13,57	0,010
arab ratıo*coat ratıo	2	53,814	53,814	26,907	38,45	0,000
Error	6	4,198	4,198	0,700		
Total	11	231,279				

S = 0,836510 R-Sq = 98,18% R-Sq(adj) = 96,67%

Table B.4 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

arab

ratıo	N	Mean	Grouping
10:00	4	64,5	A
8:02	4	57,2	B
6:04	4	56,1	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

coat

ratıo	N	Mean	Grouping
1:20	6	60,2	A
1:10	6	58,4	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

arab ratıo	coat ratıo	N	Mean	Grouping
10:00	1:20	2	65,2	A
10:00	1:10	2	63,8	A
6:04	1:20	2	59,7	B
8:02	1:10	2	58,9	B C
8:02	1:20	2	55,6	C D
6:04	1:10	2	52,6	D

Means that do not share a letter are significantly different.

Table B.5 Two way ANOVA and Tukey's comparison test for encapsulation efficiency of maltodextrin-casein combination with different ratios

Factor	Type	Levels	Values
csn ratıo	fixed	3	6:04; 8:02; 10:00
coat ratıo	fixed	2	1:10; 1:20

Analysis of Variance for eff, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
csn ratıo	2	509,24	509,24	254,62	87,14	0,000
coat ratıo	1	416,93	416,93	416,93	142,69	0,000
csn ratıo*coat ratıo	2	211,72	211,72	105,86	36,23	0,000
Error	6	17,53	17,53	2,92		
Total	11	1155,42				

S = 1,70939 R-Sq = 98,48% R-Sq(adj) = 97,22%

Unusual Observations for eff

Obs	eff	Fit	SE Fit	Residual	St Resid
3	81,5750	84,3905	1,2087	-2,8155	-2,33 R
6	87,2060	84,3905	1,2087	2,8155	2,33 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Table B.5 (Continued)

csn ratıo	N	Mean	Grouping
8:02	4	78,4	A
6:04	4	78,2	A
10:00	4	64,5	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

coat ratıo	N	Mean	Grouping
1:20	6	79,6	A
1:10	6	67,8	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

csn ratıo	coat ratıo	N	Mean	Grouping
6:04	1:20	2	89,1	A
8:02	1:20	2	84,4	A
8:02	1:10	2	72,4	B
6:04	1:10	2	67,2	B C
10:00	1:20	2	65,2	C
10:00	1:10	2	63,8	C

Means that do not share a letter are significantly different.

Table B.6 Two way ANOVA and Tukey's comparison test for encapsulation efficiency of maltodextrin-WPC combination with different ratios

Factor	Type	Levels	Values
WPC ratıo	fixed	3	6:04; 8:02; 10:00
coat ratıo	fixed	2	1:10; 1:20

Analysis of Variance for eff, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
WPC ratıo	2	192,44	192,44	96,22	77,47	0,000
coat ratıo	1	254,86	254,86	254,86	205,18	0,000
WPC ratıo*coat ratıo	2	92,05	92,05	46,02	37,05	0,000
Error	6	7,45	7,45	1,24		
Total	11	546,80				

S = 1,11449 R-Sq = 98,64% R-Sq(adj) = 97,50%

Grouping Information Using Tukey Method and 95,0% Confidence

WPC ratıo	N	Mean	Grouping
8:02	4	72,5	A
10:00	4	64,5	B
6:04	4	63,5	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Table B.6 (Continued)

coat ratıo	N	Mean	Grouping
1:20	6	71,4	A
1:10	6	62,2	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

WPC ratıo	coat ratıo	N	Mean	Grouping
8:02	1:20	2	79,3	A
6:04	1:20	2	69,8	B
8:02	1:10	2	65,6	B C
10:00	1:20	2	65,2	C
10:00	1:10	2	63,8	C
6:04	1:10	2	57,3	D

Means that do not share a letter are significantly different.

Table B.7 Two way ANOVA and Tukey's comparison test for antioxidant activity

Factor	Type	Levels	Values
ratıo	fixed	7	1; 2; 3; 4; 5; 6; 7
core coat	fixed	2	1:10; 1:20

Table B.7 (Continued)

Analysis of Variance for DPPH, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
ratıo	6	71,594	71,594	11,932	2,53	0,071
core coat	1	632,615	632,615	632,615	134,21	0,000
ratıo*core coat	6	12,480	12,480	2,080	0,44	0,839
Error	14	65,991	65,991	4,714		
Total	27	782,679				

S = 2,17108 R-Sq = 91,57% R-Sq(adj) = 83,74%

Unusual Observations for DPPH

Obs	DPPH	Fit	SE Fit	Residual	St Resid
3	27,7130	24,1970	1,5352	3,5160	2,29 R
10	20,6810	24,1970	1,5352	-3,5160	-2,29 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

ratıo	N	Mean	Grouping
2	4	19,9	A
3	4	19,2	A
1	4	19,1	A
6	4	18,0	A
7	4	17,5	A
5	4	15,8	A
4	4	15,4	A

Means that do not share a letter are significantly different.

Table B.7 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

core
coat N Mean Grouping
1:10 14 22,6 A
1:20 14 13,1 B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

core
ratıo coat N Mean Grouping
2 1:10 2 26,0 A
3 1:10 2 24,2 A
1 1:10 2 23,9 A
6 1:10 2 22,3 A B
7 1:10 2 21,5 A B C
5 1:10 2 20,6 A B C
4 1:10 2 19,6 A B C D
1 1:20 2 14,4 B C D
3 1:20 2 14,2 B C D
6 1:20 2 13,8 B C D
2 1:20 2 13,7 B C D
7 1:20 2 13,5 C D
4 1:20 2 11,1 D
5 1:20 2 11,0 D

Means that do not share a letter are significantly different.

Table B.8 Two way ANOVA and Tukey's comparison test for antioxidant activity of maltodextrin-gum arabic combination with different ratios

Factor	Type	Levels	Values
arab ratıo	fixed	3	6:04; 8:02; 10:00
coat ratıo	fixed	2	1:10; 1:20

Analysis of Variance for DPPH, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
arab ratıo	2	1,429	1,429	0,714	0,12	0,887
coat ratıo	1	338,640	338,640	338,640	57,86	0,000
arab ratıo*coat ratıo	2	4,585	4,585	2,292	0,39	0,692
Error	6	35,119	35,119	5,853		
Total	11	379,772				

S = 2,41932 R-Sq = 90,75% R-Sq(adj) = 83,05%

Unusual Observations for DPPH

Obs	DPPH	Fit	SE Fit	Residual	St Resid
9	27,7130	24,1970	1,7107	3,5160	2,06 R
12	20,6810	24,1970	1,7107	-3,5160	-2,06 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

arab ratıo	N	Mean	Grouping
6:04	4	19,9	A
8:02	4	19,2	A
10:00	4	19,1	A

Table B.8 (Continued)

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

coat
ratıo N Mean Grouping
1:10 6 24,7 A
1:20 6 14,1 B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

arab coat
ratıo ratıo N Mean Grouping
6:04 1:10 2 26,0 A
8:02 1:10 2 24,2 A
10:00 1:10 2 23,9 A B
10:00 1:20 2 14,4 B C
8:02 1:20 2 14,2 C
6:04 1:20 2 13,7 C

Means that do not share a letter are significantly different.

Table B.9 Two way ANOVA and Tukey's comparison test for antioxidant activity of maltodextrin-casein combination with different ratios

Factor	Type	Levels	Values
csn ratıo	fixed	3	6:04; 8:02; 10:00
coat ratıo	fixed	2	1:10; 1:20

Analysis of Variance for DPPH, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
csn ratıo	2	33,387	33,387	16,694	5,32	0,047
coat ratıo	1	253,994	253,994	253,994	81,01	0,000
csn ratıo*coat ratıo	2	0,723	0,723	0,362	0,12	0,893
Error	6	18,811	18,811	3,135		
Total	11	306,916				

S = 1,77066 R-Sq = 93,87% R-Sq(adj) = 88,76%

Grouping Information Using Tukey Method and 95,0% Confidence

csn ratıo	N	Mean	Grouping
10:00	4	19,1	A
8:02	4	15,8	A
6:04	4	15,4	A

Means that do not share a letter are significantly different.

Table B.9 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

coat ratıo	N	Mean	Grouping
1:10	6	21,4	A
1:20	6	12,2	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

csn ratıo	coat ratıo	N	Mean	Grouping
10:00	1:10	2	23,9	A
8:02	1:10	2	20,6	A B
6:04	1:10	2	19,6	A B
10:00	1:20	2	14,4	B C
6:04	1:20	2	11,1	C
8:02	1:20	2	11,0	C

Means that do not share a letter are significantly different.

Table B.10 Two way ANOVA and Tukey's comparison test for antioxidant activity of maltodextrin-WPC combination with different ratios

Factor	Type	Levels	Values
WPC ratıo	fixed	3	6:04; 8:02; 10:00
coat ratıo	fixed	2	1:10; 1:20

Table B.10 (Continued)

Analysis of Variance for DPPH, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
WPC ratıo	2	5,446	5,446	2,723	0,66	0,549
coat ratıo	1	226,357	226,357	226,357	55,26	0,000
WPC ratıo*coat ratıo	2	1,125	1,125	0,562	0,14	0,874
Error	6	24,579	24,579	4,096		
Total	11	257,507				

S = 2,02397 R-Sq = 90,46% R-Sq(adj) = 82,50%

Grouping Information Using Tukey Method and 95,0% Confidence

WPC ratıo	N	Mean	Grouping
10:00	4	19,1	A
6:04	4	18,0	A
8:02	4	17,5	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

coat ratıo	N	Mean	Grouping
1:10	6	22,6	A
1:20	6	13,9	B

Means that do not share a letter are significantly different.

Table B.10 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

WPC ratıo	coat ratıo	N	Mean	Grouping
10:00	1:10	2	23,9	A
6:04	1:10	2	22,3	A B
8:02	1:10	2	21,5	A B C
10:00	1:20	2	14,4	B C
6:04	1:20	2	13,7	C
8:02	1:20	2	13,5	C

Means that do not share a letter are significantly different.

Table B.11 Two way ANOVA and Tukey's comparison test for Sauter mean diameter

Factor	Type	Levels	Values
ratıo	fixed	7	1; 2; 3; 4; 5; 6; 7
core coat	fixed	2	1:10; 1:20

Analysis of Variance for dıameter, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
ratıo	6	266,070	266,070	44,345	870,55	0,000
core coat	1	62,372	62,372	62,372	1224,43	0,000
ratıo*core coat	6	30,742	30,742	5,124	100,59	0,000
Error	14	0,713	0,713	0,051		
Total	27	359,897				

S = 0,225697 R-Sq = 99,80% R-Sq(adj) = 99,62%

Table B.11 (Continued)

Unusual Observations for dıameter

Obs	dıameter	Fit	SE Fit	Residual	St Resid
18	5,0400	5,3800	0,1596	-0,3400	-2,13 R
25	5,7200	5,3800	0,1596	0,3400	2,13 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

ratıo	N	Mean	Grouping
2	4	11,0	A
1	4	10,5	A
3	4	8,0	B
4	4	6,4	C
7	4	4,3	D
5	4	3,9	D
6	4	2,3	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

core coat	N	Mean	Grouping
1:10	14	8,1	A
1:20	14	5,1	B

Means that do not share a letter are significantly different.

Table B.11 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

ratıo	core coat	N	Mean	Grouping
1	1:10	2	14,2	A
2	1:10	2	13,2	B
2	1:20	2	8,7	C
3	1:10	2	8,4	C D
3	1:20	2	7,6	D E
4	1:10	2	7,4	E
1	1:20	2	6,8	E
5	1:10	2	5,4	F
4	1:20	2	5,4	F
7	1:10	2	5,2	F
7	1:20	2	3,4	G
6	1:10	2	3,0	G H
5	1:20	2	2,4	H I
6	1:20	2	1,7	I

Means that do not share a letter are significantly different.

Table B.12 Two way ANOVA and Tukey's comparison test for span

Factor	Type	Levels	Values
ratıo	fixed	7	1; 2; 3; 4; 5; 6; 7
core coat	fixed	2	1:10; 1:20

Table B.12 (Continued)

Analysis of Variance for span, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
ratıo	6	153,351	153,351	25,558	389,22	0,000
core coat	1	31,017	31,017	31,017	472,35	0,000
ratıo*core coat	6	20,195	20,195	3,366	51,26	0,000
Error	14	0,919	0,919	0,066		
Total	27	205,482				

S = 0,256254 R-Sq = 99,55% R-Sq(adj) = 99,14%

Unusual Observations for span

Obs	span	Fit	SE Fit	Residual	St Resid
2	2,7090	3,0935	0,1812	-0,3845	-2,12 R
9	3,4780	3,0935	0,1812	0,3845	2,12 R
16	3,4560	2,9655	0,1812	0,4905	2,71 R
23	2,4750	2,9655	0,1812	-0,4905	-2,71 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

ratıo	N	Mean	Grouping
6	4	9,2	A
7	4	6,9	B
5	4	6,8	B
4	4	5,9	C
3	4	4,1	D
2	4	3,0	E
1	4	2,0	F

Table B.12 (Continued)

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

core

coat	N	Mean	Grouping
1:20	14	6,5	A
1:10	14	4,4	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

core

ratıo	coat	N	Mean	Grouping
6	1:20	2	11,3	A
7	1:20	2	8,9	B
5	1:20	2	8,8	B
6	1:10	2	7,2	C
4	1:20	2	6,5	C
4	1:10	2	5,2	D
7	1:10	2	4,9	D
5	1:10	2	4,8	D
3	1:20	2	4,4	D E
3	1:10	2	3,8	E F
2	1:10	2	3,1	F G
2	1:20	2	3,0	F G
1	1:20	2	2,4	G H
1	1:10	2	1,5	H

Means that do not share a letter are significantly different.

Table B.13 Two way ANOVA and Tukey's comparison test for specific surface area

Factor	Type	Levels	Values
ratıo	fixed	7	1; 2; 3; 4; 5; 6; 7
core coat	fixed	2	1:10; 1:20

Analysis of Variance for area, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
ratıo	6	15,1025	15,1025	2,5171	4463,02	0,000
core coat	1	3,4860	3,4860	3,4860	6181,01	0,000
ratıo*core coat	6	2,0101	2,0101	0,3350	594,00	0,000
Error	14	0,0079	0,0079	0,0006		
Total	27	20,6065				

S = 0,0237484 R-Sq = 99,96% R-Sq(adj) = 99,93%

Grouping Information Using Tukey Method and 95,0% Confidence

ratıo	N	Mean	Grouping
6	4	2,8	A
5	4	1,8	B
7	4	1,5	C
4	4	1,0	D
3	4	0,7	E
1	4	0,7	F
2	4	0,6	F

Means that do not share a letter are significantly different.

Table B.13 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

core coat	N	Mean	Grouping
1:20	14	1,7	A
1:10	14	0,9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

ratıo	core coat	N	Mean	Grouping
6	1:20	2	3,6	A
5	1:20	2	2,5	B
6	1:10	2	2,0	C
7	1:20	2	1,8	D
4	1:20	2	1,2	E
7	1:10	2	1,1	E
5	1:10	2	1,1	E
1	1:20	2	0,9	F
4	1:10	2	0,8	G
2	1:20	2	0,8	G
3	1:20	2	0,8	G H
3	1:10	2	0,7	H
2	1:10	2	0,5	I
1	1:10	2	0,4	I

Means that do not share a letter are significantly different.

Table B.14 Two way ANOVA and Tukey's comparison test for sauter mean diameter of maltodextrin-gum arabic combination with different ratios

Factor	Type	Levels	Values
coat ratıo	fixed	2	1:10; 1:20
arab ratıo	fixed	3	6:04; 8:02; 10:00

Analysis of Variance for SIZE, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
coat ratıo	1	53,425	53,425	53,425	1329,54	0,000
arab ratıo	2	19,790	19,790	9,895	246,24	0,000
coat ratıo*arab ratıo	2	21,320	21,320	10,660	265,29	0,000
Error	6	0,241	0,241	0,040		
Total	11	94,776				

S = 0,200458 R-Sq = 99,75% R-Sq(adj) = 99,53%

Grouping Information Using Tukey Method and 95,0% Confidence

coat ratıo	N	Mean	Grouping
1:10	6	11,9	A
1:20	6	7,7	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Table B.14 (Continued)

arab ratıo	N	Mean	Grouping
6:04	4	11,0	A
10:00	4	10,5	B
8:02	4	8,0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

coat ratıo	arab ratıo	N	Mean	Grouping
1:10	10:00	2	14,2	A
1:10	6:04	2	13,2	B
1:20	6:04	2	8,7	C
1:10	8:02	2	8,4	C
1:20	8:02	2	7,6	D
1:20	10:00	2	6,8	E

Means that do not share a letter are significantly different.

Table B.15 Two way ANOVA and Tukey's comparison test for sauter mean diameter of maltodextrin-casein combination with different ratios

Factor	Type	Levels	Values
csn ratıo	fixed	3	6:04; 8:02; 10:00
coat ratıo	fixed	2	1:10; 1:20

Table B.15 (Continued)

Analysis of Variance for SIZE, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
csn ratıo	2	87,229	87,229	43,614	505,18	0,000
coat ratıo	1	51,253	51,253	51,253	593,67	0,000
csn ratıo*coat ratıo	2	15,953	15,953	7,976	92,39	0,000
Error	6	0,518	0,518	0,086		
Total	11	154,953				

S = 0,293825 R-Sq = 99,67% R-Sq(adj) = 99,39%

Grouping Information Using Tukey Method and 95,0% Confidence

csn ratıo	N	Mean	Grouping
10:00	4	10,5	A
6:04	4	6,4	B
8:02	4	3,9	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

coat ratıo	N	Mean	Grouping
1:10	6	9,0	A
1:20	6	4,9	B

Means that do not share a letter are significantly different.

Table B.15 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

csn ratıo	coat ratıo	N	Mean	Grouping
10:00	1:10	2	14,1	A
6:04	1:10	2	7,4	B
10:00	1:20	2	6,8	B
8:02	1:10	2	5,4	C
6:04	1:20	2	5,4	C
8:02	1:20	2	2,4	D

Means that do not share a letter are significantly different.

Table B.16 Two way ANOVA and Tukey's comparison test for sauter mean diameter of maltodextrin-WPC combination with different ratios

Factor	Type	Levels	Values
WPC ratıo	fixed	3	6:04; 8:02; 10:00
coat ratıo	fixed	2	1:10; 1:20

Analysis of Variance for sıze, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
WPC ratıo	2	143,831	143,831	71,915	3072,21	0,000
coat ratıo	1	36,995	36,995	36,995	1580,44	0,000
WPC ratıo*coat ratıo	2	22,212	22,212	11,106	474,45	0,000
Error	6	0,140	0,140	0,023		
Total	11	203,178				

Table B.16 (Continued)

S = 0,152998 R-Sq = 99,93% R-Sq(adj) = 99,87%

Grouping Information Using Tukey Method and 95,0% Confidence

WPC ratıo	N	Mean	Grouping
10:00	4	10,5	A
8:02	4	4,3	B
6:04	4	2,3	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

coat ratıo	N	Mean	Grouping
1:10	6	7,5	A
1:20	6	4,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

WPC ratıo	coat ratıo	N	Mean	Grouping
10:00	1:10	2	14,2	A
10:00	1:20	2	6,8	B
8:02	1:10	2	5,2	C
8:02	1:20	2	3,4	D
6:04	1:10	2	3,0	D
6:04	1:20	2	1,7	E

Means that do not share a letter are significantly different.

Table B.17 Two way ANOVA and Tukey's comparison test for total phenolic content of maltodextrin-casein combination with different ratios at 80°C for different time durations

Factor	Type	Levels	Values
ratıo	fixed	2	6:04; 8:02
Hour	fixed	6	0,0; 1,0; 2,0; 3,0; 3,5; 4,0

Analysis of Variance for TPC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
ratıo	1	7131282	7131282	7131282	76,89	0,000
Hour	5	95297789	95297789	19059558	205,49	0,000
ratıo*Hour	5	31522553	31522553	6304511	67,97	0,000
Error	12	1113004	1113004	92750		
Total	23	135064628				

S = 304,549 R-Sq = 99,18% R-Sq(adj) = 98,42%

Unusual Observations for TPC

Obs	TPC	Fit	SE Fit	Residual	St Resid
6	16030,1	15503,2	215,3	526,9	2,45 R
18	14976,3	15503,2	215,3	-526,9	-2,45 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

ratıo	N	Mean	Grouping
8:02	12	15318,4	A
6:04	12	14228,2	B

Table B.17 (Continued)

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Hour	N	Mean	Grouping
1,0	4	17347,3	A
0,0	4	17258,2	A
2,0	4	15212,9	B
3,0	4	13497,8	C
3,5	4	13040,9	C
4,0	4	12282,8	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

ratıo	Hour	N	Mean	Grouping
6:04	0,0	2	18380,1	A
6:04	1,0	2	18051,6	A
8:02	1,0	2	16643,0	B
8:02	0,0	2	16136,4	B C
8:02	2,0	2	15503,2	B C D
8:02	3,0	2	15363,4	C D
6:04	2,0	2	14922,6	D
8:02	3,5	2	14610,8	D E
8:02	4,0	2	13653,8	E
6:04	3,0	2	11632,3	F
6:04	3,5	2	11471,0	F
6:04	4,0	2	10911,8	F

Means that do not share a letter are significantly different.

Table B.18 Two way ANOVA and Tukey's comparison test for total phenolic content of maltodextrin-WPC combination with different ratios at 80°C for different time durations

Factor	Type	Levels	Values
Ratıo	fixed	2	6:04; 8:02
Hour	fixed	6	0,0; 1,0; 2,0; 3,0; 3,5; 4,0

Analysis of Variance for TPC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Ratıo	1	50843206	50843206	50843206	820,01	0,000
Hour	5	115290309	115290309	23058062	371,88	0,000
Ratıo*Hour	5	17349350	17349350	3469870	55,96	0,000
Error	12	744040	744040	62003		
Total	23	184226904				

S = 249,005 R-Sq = 99,60% R-Sq(adj) = 99,23%

Unusual Observations for TPC

Obs	TPC	Fit	SE Fit	Residual	St Resid
2	17974,2	18334,4	176,1	-360,2	-2,05 R
8	15298,9	14911,8	176,1	387,1	2,20 R
14	18694,6	18334,4	176,1	360,2	2,05 R
20	14524,7	14911,8	176,1	-387,1	-2,20 R

R denotes an observation with a large standardized residual.

Table B.18 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

Ratıo	N	Mean	Grouping
8:02	12	15561,1	A
6:04	12	12650,1	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Hour	N	Mean	Grouping
0,0	4	18644,4	A
1,0	4	14594,6	B
2,0	4	13831,2	C
3,0	4	12981,7	D
3,5	4	12460,2	D E
4,0	4	12121,5	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Ratıo	Hour	N	Mean	Grouping
6:04	0,0	2	18954,5	A
8:02	0,0	2	18334,4	A
8:02	1,0	2	16600,0	B
8:02	2,0	2	16094,6	B
8:02	3,0	2	14911,8	C
8:02	3,5	2	14019,4	C D
8:02	4,0	2	13406,5	D E
6:04	1,0	2	12589,2	E
6:04	2,0	2	11567,7	F

Table B.18 (Continued)

6:04	3,0	2	11051,6	F
6:04	3,5	2	10901,1	F
6:04	4,0	2	10836,6	F

Means that do not share a letter are significantly different.

Table B.19 One way ANOVA and Tukey's comparison test for total phenolic content of phenolic powder without coating at 80°C for different time durations

Source	DF	SS	MS	F	P
hour	5	690592350	138118470	422,59	0,000
Error	6	1961042	326840		
Total	11	692553392			

S = 571,7 R-Sq = 99,72% R-Sq(adj) = 99,48%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev
0,0	2	51087	1287
1,0	2	27650	178
2,0	2	31741	255
3,0	2	34659	255
3,5	2	34299	357
4,0	2	30389	127

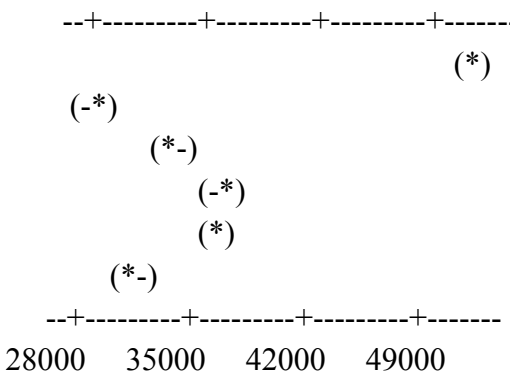

Table B.19 (Continued)

Pooled StDev = 572

Grouping Information Using Tukey Method

hour	N	Mean	Grouping
0,0	2	51087	A
3,0	2	34659	B
3,5	2	34299	B
2,0	2	31741	C
4,0	2	30389	C
1,0	2	27650	D

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of hour

Individual confidence level = 99,27%

hour = 0,0 subtracted from:

hour	Lower	Center	Upper	------+---------+---------+---------+---
1,0	-25713	-23437	-21161	(--*-)
2,0	-21622	-19347	-17071	(--*-)
3,0	-18704	-16428	-14152	(--*-)
3,5	-19064	-16788	-14512	(-*-)
4,0	-22974	-20698	-18422	(-*--)
				------+---------+---------+---------+---
				-20000 -10000 0 10000

Table B.19 (Continued)

hour = 1,0 subtracted from:

hour	Lower	Center	Upper	------+---------+---------+---------+---
2,0	1814	4090	6366	(-*-)
3,0	4733	7009	9285	(-*-)
3,5	4373	6649	8925	(--*-)
4,0	463	2739	5015	(--*-)
				------+---------+---------+---------+---
				-20000 -10000 0 10000

hour = 2,0 subtracted from:

hour	Lower	Center	Upper	------+---------+---------+---------+---
3,0	643	2919	5195	(-*-)
3,5	283	2559	4835	(--*-)
4,0	-3627	-1351	925	(--*-)
				------+---------+---------+---------+---
				-20000 -10000 0 10000

hour = 3,0 subtracted from:

hour	Lower	Center	Upper	------+---------+---------+---------+---
3,5	-2636	-360	1916	(--*-)
4,0	-6546	-4270	-1994	(--*-)
				------+---------+---------+---------+---
				-20000 -10000 0 10000

hour = 3,5 subtracted from:

hour	Lower	Center	Upper	------+---------+---------+---------+---
4,0	-6186	-3910	-1634	(-*-)
				------+---------+---------+---------+---
				-20000 -10000 0 10000