ENCAPSULATION OF WHEAT GERM OIL

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ABSTRACT

ENCAPSULATION OF WHEAT GERM OIL

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Wheat germ oil is a rich source of omega 3 and omega 6, octacosanol and tocopherol which has vitamin E activity. Due to these properties it is beneficial for health but it is prone to oxidation in free form. The aim of this study was to encapsulate wheat germ oil in micron size and determine the best encapsulation conditions by analysing encapsulation efficiency, particle size distribution and surface morphology of the capsules.

The effects of core to coating ratio, coating materials ratio and ultrasonication time on encapsulation of wheat germ oil were investigated. Maltodextrin (MD) and whey protein concentrate (WPC) at different ratios (3:1, 2:2, 1:3) were used as coating materials. Total solid content of all samples was 40% (w/w). Five different core to coating ratios (1:8, 1:4, 2:4, 3:4, 4:4) were experimented. Ultrasound was used at 320 W and 20 kHz frequency for three different times (2, 5, 10 min). Prepared emulsions were frozen and then freeze dried for 48 hours to obtain microcapsules. Encapsulation efficiency analysis, particle size analysis and scanning electron microscopy (SEM) analysis were performed.

Increasing WPC content in coating led to an increase in encapsulation efficiency. Microcapsules prepared with MD:WPC ratio of 1:3 were found to have higher encapsulation efficiencies (65.62%-89.62%) than the other ratios. Increase in oil load led to decrease in encapsulation efficiency thus 1:8 core to coating ratio gave better results. The best conditions for microcapsules were determined as ultrasonication time 10 min, core to coating ratio of 1:8 and MD:WPC ratio 1:3.

Keywords: microencapsulation, wheat germ oil, ultrasonication, encapsulation efficiency

ÖZ

RUŞEYM YAĞININ ENKAPSÜLASYONU

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Yüksek Lisans, Gıda Mühendisliği Bölümü
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Ruşeym yağı omega 3 ve omega 6, oktakosanol ve E vitamini aktivitesi gösteren tokoferol açısından zengindir. Bu özelliklerinden dolayı sağlığa faydalıdır fakat serbest haldeyken oksitlenmeye oldukça müsaittir. Bu çalışmanın amacı ruşeym yağını mikron boyutunda kaplamak ve kaplama verimi, parçacık boyutu dağılımı ve yüzey morfolojisini analiz ederek en iyi kaplama koşulunu belirlemektir.

Yağın kaplama maddesine oranı, kaplama maddelerinin birbirine oranı ve ultrason uygulama zamanının ruşeym yağını kaplamaya olan etkileri araştırılmıştır. Farklı oranlarda (3:1, 2:2, 1:3) karıştırılan maltodekstrin ve peynir altı suyu proteini, kaplama maddesi olarak kullanılmıştır. Bütün kaplama maddesi karışımlarında toplam katı madde miktarı ağırlıça %40. Yağ:kaplama maddesi oranı olarak 1:8, 1:4, 2:4, 3:4, 4:4 şeklinde beş farklı oran denenmiştir. Ultrason 320 W gücünde ve 20 kHz frekansta 2 dak, 5 dak ve 10 dak çalıştırılmıştır. Mikrokapsül elde edebilmek için hazırlanmış emülsiyonlar dondurulduktan sonra dondurmalı kurutucuda 48 saat kurutulmuştur. Kaplama verimi analizi, parçacık boyutu analizi ve SEM analizi yapılmıştır.

Kaplama maddesinde peynir altı suyu konsantresinin oranının artması kaplama veriminde artışa sebep olmuştur. Kaplama maddesi karışımında MD:WPC oranı 1:3olan kapsüllerin enkapsülasyon verimlerinin daha yüksek olduğu (%65,62-%89,62) bulunmuştur. Mikrokapsülde yağ oranındaki artış kaplama veriminde azalmaya neden olmuştur dolayısıyla 1:8 yağ:kaplama maddesi oranı daha iyi sonuç vermiştir. Ultrason uygulama süresinin 10 dak olduğu, kaplama maddesi karışımında MD:WPC oranının 1:3 olduğu ve yağ:kaplama maddesi oranının 1:8 olduğu koşullar mikrokapsül hazırlamak için en uygun koşullar olarak belirlenmiştir.

Anahtar Kelimeler: mikroenkapsülasyon, ruşeym yağı, ultrasonikasyon, enkaspülasyon verimi

To my family

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CHAPTER 1

INTRODUCTION

1.1. Functional Foods

The term ‘‘functional food’’ is generally used for food products which are fortified with additional special ingredients that have health benefits. The idea of functional food was first emerged in Japan, in the 1980s (Stanton et al., 2005).

There are different definitions for functional food because it can not be limited to a certain group of food products. Definition agreed by The European Commision’s Concerted Action on Functional Food Science in Europe (FuFoSE) is: A food product can be regarded as functional food if it has beneficial effect on one or more functions of body or it has the ability to reduce risk of a disease. Also a functional food is not a pill, it is in the form of normal food (Diplock et al., 1999).

Functional foods may help to improve physical endurance of the body, reduce the risk of some illnesses (e.g. foods rich in antioxidants for prevention of cancer), and could even be used for cure of some diseases. Public surveys revealed that there is a public demand for functional food because it has the potential to prevent diseases and has a positive effect on health. Due to these health benefits, it can reduce the medical service expenses (Mark-Herbert, 2004; Menrad, 2003).

1.2. Wheat Germ Oil

1.2.1. Structure of wheat grain

The wheat grain (the fruit of the cereal) contains a single seed. Pericarp which is the outermost layer of a seed, surrounds the entire seed and forms a protective covering. Testa or seed coat lies under pericarp and this tissue is directly adhered to the seed. The whole grain consists of two parts, which are the embryo and the endosperm. Endosperm is the starchy part of the grain and it serves as a storage reserve that is used by the embryo at germination. Starch that endosperm contains is used as a food source in the growth period of seedling. The aleurone layer is the outer part of the endosperm. Structure of the aleurone layer is different from inner part of endosperm because it is single layered and shape of cells are cubic. The aleurone layer is rich in nutrients such as proteins, minerals, some of B vitamins (Buri et al., 2004; Hemery et al., 2011; Pomeranz, 1988). The embryo or germ is the reproductive part of the wheat grain. It is comprised of embryo axis and scutellum. The embryo axis develops into the first roots and shoots of the new plant. The scutellum is a layer that enables connection between the embryo and the endosperm, and during germination hydrolyzed sugars are removed from endosperm to the embryo by the help of scutellum. For

different cereal varieties, location of the germ changes. It is either surrounded by the endosperm or placed laterally.

Briefly, the wheat grain is constituted by three distinct parts: the germ (embryo), the bran and the mealy endosperm. Wheat grains consists of 2–3% germ, 13–17% bran and 80–85% starchy endosperm (in dry matter basis). A sketch of the lengthwise and histological section of a wheat grain is shown in Figure 1.1.

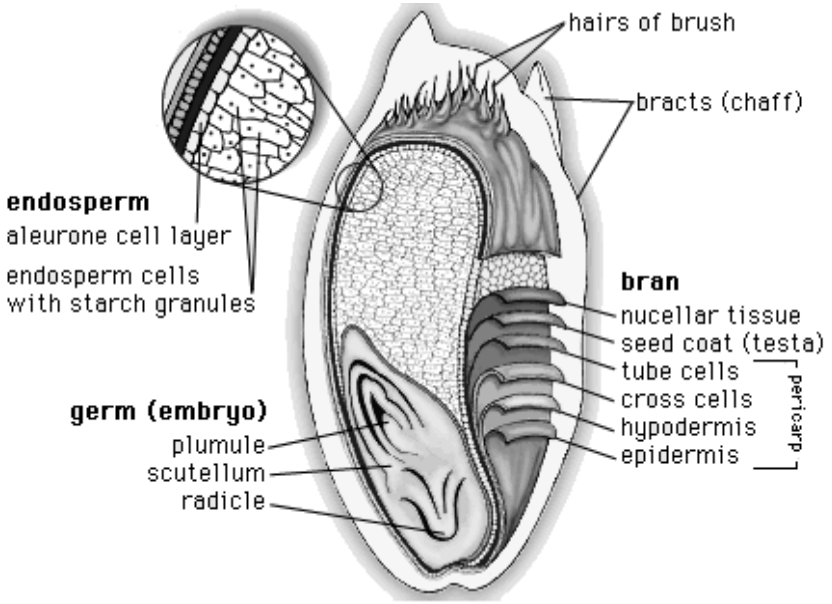

Figure 1.1 Wheat grain (Encyclopaedia Britannica, http://www.britannica.com)

Wheat is one of the leading grain crops produced, consumed, and traded worldwide. About 590 million metric tons of wheat is produced globally each year. China, India and the United States, are among the largest growers of wheat (FAOSTAT, 2010). Production of wheat in different countries in 2010 is shown in Figure 1.2. Statistical data on wheat germ production is not readily available. However, it can be estimated that about 10 million tons of wheat germ could be obtained from wheat milling operations worldwide based on the fact that about 2% of the whole wheat grain is comprised of germ.

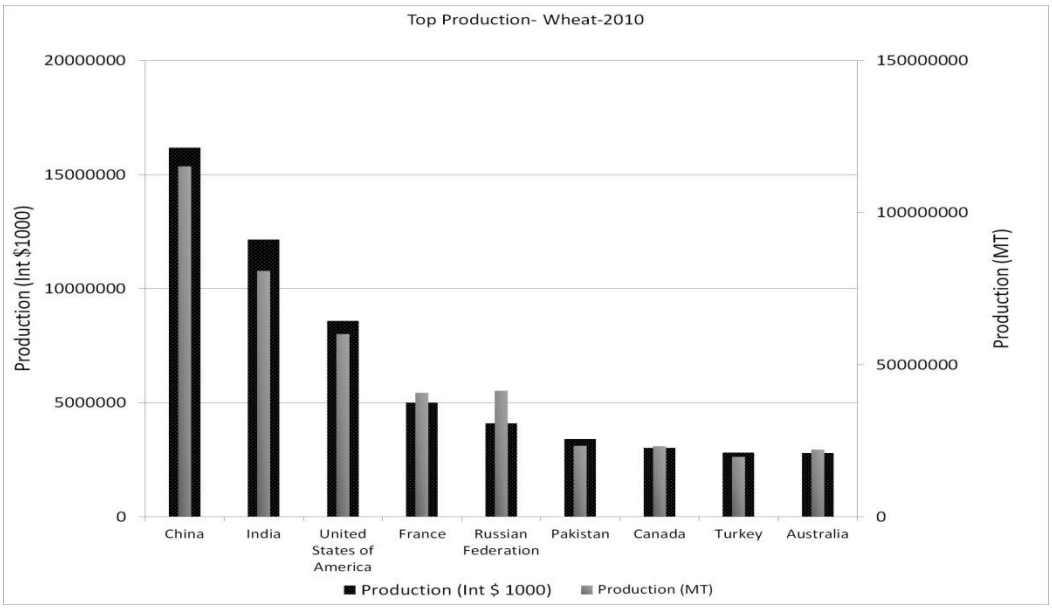

Figure 1.2 Production of wheat by countries 2010 values (FAOSTAT,2010)

World wheat consumption is increasing in recent years. The main reason for this increase is the usage of wheat for bioethanol production and animal feed.

Wheat production in Turkey is important not only for economical reasons but also for social reasons since most of the farmers cultivates wheat. Production of wheat in Turkey between 2005 and 2011 are given in Table 1.1. In Turkey nearly one third of cultivated areas are used for wheat production and wheat is the most produced grain according to data of TUİK (2011).

1.2.2. Properties of wheat germ and its recovery

Wheat germ represents 2-3% of the entire wheat grain and it contains between 8 and 14% of oil (Sonntag, 1979; Pomeranz, 1988). After wheat milling process it is separated as a side product. It is used mainly as forage and as a resource which oil is extracted. Wheat germ has high nutritional value. Average composition of wheat germ is given in Table 1.2. When germ is compared with wheat flour in terms of nutrient content it is seen that it contains higher amounts of protein, sugar and minerals (Rao et al., 1980). The protein content of wheat germ is about 30% (w/w). Wheat germ is one of the richest natural sources of α-tocopherol, which has high Vitamin E activity (Dunford, 2001).

Table 1.1 Production of wheat in Turkey (TUİK, 2011)

Year	Cultivated Area (decares)	Production (tonnes)	Yield (kg/decare)
2005	92 500 000	21 500 000	232
2006	84 900 000	20 010 000	236
2007	80 977 000	17 234 000	213
2008	80 900 000	17 782 000	220
2009	81 000 000	20 600 000	254
2010	81 034 000	19 674 000	243
2011	80 960 000	21 800 000	269

Table 1.2 Proximate composition of wheat germ (Barnes, 1982)

Compund	**Composition (%)**
Protein	2.6
Crude Fiber	3
Starch	20
Sugars	16
Oil	10
Moisture	6
Ash	4

In a wheat milling process, wheat is transferred from elevator to screen room to separate out dust, stones, mud balls, glass, nonferrous metal and grains other than wheat. After that tempering which can be defined as addition of moisture to wheat takes place. Purpose of tempering is making wheat grain softer and by that way processing wheat easily. Tempered wheat is then milled. The milling process involves grinding of the grain and fractination of wheat.

While grinding, germ tends to break into coarser pieces than bran and endosperm particles. The reason for this condition is that it contains high level of oil which gives plastic texture to germ. Germ also tends to flatten, rather than crush like endosperm. Due to these properties germ is separated by size. The density of germ is greater than that of the other wheat fractions. Thus, wheat germ can be isolated based on specific gravity differences (Dunford, 2005).

1.2.3. Wheat germ oil production

Wheat germ oil (WGO) can be extracted by mechanical pressing, organic solvent extraction, supercritical fluid extraction and pressurized solvent extraction. Hexane is commonly used solvent for WGO extraction (Anonymous, 2002). Ethanol and 1,2-dichloroethane are also used for WGO extraction but not as widely as hexane (Barnes, 1983).

Supercritical fluid extraction technology is investigated by various researchers as an alternative method to conventional hexane extraction (Panfili et al., 2003; Dunford and Martinez, 2003; Eisenmenger and Dunford, 2008). In the study of Eisenmenger and Dunford (2008), it was found that tocopherol content of oil that was obtained by supercritical CO_2 extraction was higher than that of hexane extracted oil. The phospholipid content of supercritical CO_2 extracted oil was very low. Due to this low phospholipid content in the wheat germ oil refining process, degumming step can be eliminated when supercritical CO_2 extraction method is used. Oil extracted with supercritical CO_2 has a lighter color and contains less phosphorus than that of hexane-extracted oil. Although ground and flaked wheat germ were not significantly different in terms of oil extraction rates, the use of flaked wheat germ was recommended for large-scale supercritical CO_2 extraction. According to Dunford and Martinez (2003) the α- and β-tocopherols amount found in oil were nearly same for both supercritical CO_2 extracted and hexane-extracted oil. However, Gomez and Ossa (2000) found that tocopherol content in the supercritical CO_2 extracted WGO was higher when compared the hexane-extracted oil.

Pressurized solvent extraction is another method that is used for extraction of WGO. The working principle of pressurized solvent extraction is based on utilization of organic and/or aqueous solvents at higher temperatures and pressures. Higher temperature accelerates the extraction rate, while elevated pressure prevents boiling of solvent at temperatures above the normal boiling point. In their study, Dunford and Zhang (2003) found that using pressurized solvent extraction led to a decrease in solvent consumption and extraction time with having no affect on the extraction yield and fatty acid compositon of the oil in comparison to soxhlet extraction.

1.2.4. Physicochemical properties and lipid composition of WGO

Physicochemical properties of WGO are summarized in Table 1.3. Free fatty acid (FFA) content of WGO is usually less than 6%; but if processing conditions are not controlled properly free fatty acid content can increase. Solvent-extracted crude oil usually has a lower FFA content than that of the mechanically expelled oil. As free fatty acids causes bitter and soapy flavor in oil; hence, they

have to be removed during the edible oil-refining process. Unsaponifiable matter content of WGO is higher, than that of the most other edible oils.

Table 1.3 Physicochemical properties of WGO (Firestone, 1999)

Property	
Specific Gravity	0.928–0.938 (15.5 °C) 0.925-0.933 (25.5 °C)
Refractive Index	1.474–1.483 (25 °C) 1.469-1.478 (40°C)
Iodine Value	115-128
Saponification Value	179-190
Unsaponifiable (%)	2-5

1.2.5. Utilization areas of WGO

Wheat germ oil is well known for its beneficial health effects due to its high content of vitamin E and polyunsaturated fatty acids, mainly linoleic acid (omega 6, between 44 and 65%) and linolenic acid (omega 3, in a lower proportion, 4-11%) (Wang and Jonson, 2001; Megahad and El Kinawy, 2002). The oil has a beneficial effect on the immune and endocrine systems. It stimulates reproductive functions, helps the recovery of wound and burn , reduces the cholesterol level in blood, and protects cardio (Vishnyakov, et al., 2001). Wheat germ oil is used in foods, pharmaceuticals and cosmetic formulations also as food supplement (Kahlon, 1989). Tocopherols found in wheat germ oil protect it against deterioration caused by oxidation and they perform an important biological activity as vitamin E. However, high content of polyunsaturated fatty acids makes wheat germ oil highly prone to oxidation. Thus, it can undergo transformations that may affect both its nutritional and organoleptic qualities.

1.3. Microencapsulation

Microencapsulation is the coating of small particles of solid, liquid or gas, generally called as the core or active, with another material, also known as the coating or wall material, to form small capsules (Augustin and Hemar, 2009). Different structures of encapsulated ingredients are represented in Figure 1.3. Encapsulation protects sensitive food ingredients (e.g. flavours, polyunsaturated oils, vitamins) against heat, moisture and pH until they are required to be released. In addition, microencapsulation can mask off odors of food materials and by that way utilization becomes easier.It has been shown that oxidation of materials that are susceptible to oxidation has been retarded significantly by microencapsulation (Anandaraman and Reineccius 1986; Beatus et al., 1984; Reineccius, 1994).

In general, microencapsulation process consists of two stages as: emulsion preparation and drying.

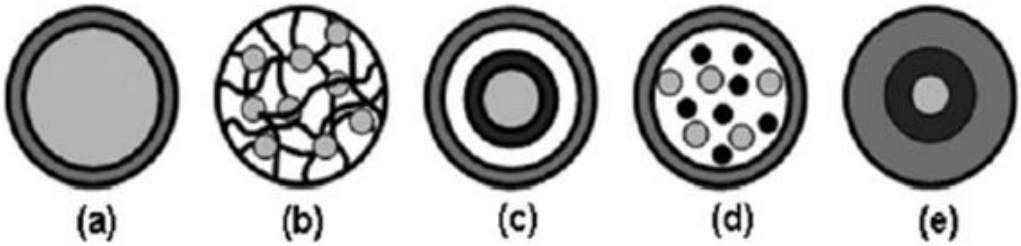

Figure 1.3 Morphologies of microcapsules : (a) single-core capsule, (b) dispersed core in polymer gel, (c) multi-layer capsule, (d) dual core capsule and (e) single-core-multi-shell capsule

1.3.1. Emulsion Preparation

Emulsification is one of the steps in encapsulation of bioactive compounds. An emulsion is a dispersion of two liquids that normally do not mix (e.g. oil, water) in each other. In an emulsion system, one of the liquids is dispersed in another in the form of fine droplets. Emulsion droplet size affects emulsion properties like rheology, stability, appearance and shelf life (Becher, 2001; McClements, 2005).

Properties of emulsion are important because they affect the retention of volatiles and also surface oil amount of the final encapsulated powder (Jafari et al., 2008). In emulsion preparation, ultrasonication or microfluidization can be used.

1.3.1.1. Ultrasonication

A sonicator mainly consists of a generator, a converter and a probe. Generator converts alternating electrical current to high frequency electrical energy. The convertor transforms this energy to mechanical vibrations and transmits this motion to the probe (Jafari et al., 2007). Two mechanisms are responsible for ultrasonic emulsification. For an oil-water mixture; firstly, sonicator creates an acoustic field that produces interfacial waves and this results in the dispersion of the oil phase into the water medium in the form of very small droplets (Li and Fogler, 1978). Secondly, when low frequency ultrasound is applied to the liquid, sound wave transmitted to the system causes pressure fluctuations and this creates cavitation which is formation and immediate collapse of bubbles which are micron size. Collapse of these bubbles creates very strong turbulences. Accumulation of many thousands of these miniature implosions form the basis of ultrasonic homogenization. The turbulence caused by microbubbles break up bigger droplets of dispersed phase into smaller ones. Among these two mechanisms, cavitation was found to be the main phenomenon responsible for effects that are caused by sonicator (Jayasooriya et al., 2004).

Using ultrasound has advantages like lowering energy consumption and production of a more homogeneous emulsion in comparison to mechanical process (Abismail et al.,1999). In addition, stable emulsions even without the addition of surfactant were found to be generated with ultrasound (Mason et al., 1996).

Klaypradit and Huang (2007) used ultrasonic atomizer and they used three steps for encapsulation of tuna oil: emulsification, ultrasonic atomization and freeze drying. They found that ultrasound technology used in this study increased the stability of tuna oil and it can be applied to industrial scale. Ultrasonic or ultra-turrax treatments were compared in terms of emulsion stability and encapsulation efficiency by Mongenot et al. (2000). It was found that ultrasonic emulsification resulted in a lower microcapsule size and in higher aroma retention (94.3%) than ultra turrax (83.3%) .

Kentish et al. (2007) prepared food grade emulsions from a mixture of flaxseed oil and water by using ultrasonication for batch and continuous operation. Results showed that increasing ultrasonication time reduced droplet sizes down to 5 min but continued sonication beyond five min did not have an effect on droplet size. When batch and continuous operations were compared, it was observed that while the batch equipment produced better results, continuous equipment was more common to be used in industry for commercial purposes.

In their study, Cilek et al. (2012) used ultrasonication for emulsion preparation in microencapsulation of phenolic compounds derived from pomace of sour cherry. Maltodextrin and gum arabic were used as wall materials in this study. Effect of different ultrasonication times in particle size was investigated. It was found that increasing ultrasonication time decreased particle size down to a certain amount and then particle size became constant. Surface phenolic content of samples prepared at five and ten minutes were found to be higher when compared to samples prepared at 15, 20 and 25 min.

1.3.1.2. Microfluidization

Microfluidization is a high pressure homogenization system that is used to make stable emulsions that have small emulsion droplet size. In microfluidizer, flow stream is guided by high pressure which is created by a pneumatically powered pump that can compress air up to about 150 MPa through a fine orifice. Two streams flow opposite to each other and they collide with one another in the interaction chamber of microfluidizer (Olson et al., 2004; Schultz et al., 2004). This collision creates shear forces and cavitation which lead to a decrease in droplet size of emulsion (Maa and Hsu, 1999).

Emulsification by microfluidization has been found to be more advantageous than other conventional homogenizers by various researchers. The particle size distributions of emulsions produced by a microfluidizer found to be smaller and shorter spanned than the products of traditional homogenization (Pinnamani et al., 2003; Robin et al., 1993; Dalgleish et al., 1996; McCrae, 1994). But , microfluidization may cause "over-processing" . Over processing is a phenomenon that is defined as re-coalescence of emulsion droplets and as a result of re-coalescence, an increase in droplet size of emulsion. Over-processing occurs at certain conditions such as higher pressures and longer emulsification times (Jafari et al., 2006, 2007; Lobo and Sverika, 2003; Olson et al., 2004).

In their study, Jafari et al. (2006) compared ultrasonication and microfluidization for nano-emulsion production of d-limonene. Results of the study showed that by using both methods, nano emulsions that had similar particle size range were produced. Size distributions of emulsions that were produced with microfluidizer was narrower and sonication was easier to use in terms of operation and cleaning.

1.3.2. Drying

Prepared emulsions are dried using different methods to obtain capsules.

1.3.2.1. Spray drying

Spray drying is a commonly used drying method in many sectors of the food industry. It is a preferred encapsulation method because of its lower cost than other techniques. Spray drying is generally used for the production of many encapsulated food ingredients vitamins, minerals, flavours, oils, enzymes and probiotic microorganisms. The main steps in the production of a spray dried encapsulated ingredient involve dissolving the core material in a dispersion of the wall material and atomising the dispersion into hot air medium in the drying chamber to enable rapid removal of water. At the outlet of spray drier, powder particles are separated. It is notable that spray drying may be used for heat sensitive and volatile ingredients (e.g. flavours) as the wall material protects the core and limits losses of volatiles. This is because of the short exposure time

to the hot air in the dryer and rapid water evaporation which keeps the temperature of the core low. Flavors of oregano, citronella and sweet marjoram were encapsulated with whey protein concentrate and skimmed milk powder separately by using spray drying. Results of the study showed that spherical shaped microcapsules were obtained and encapsulation efficiency varied between 50%- 80%.

1.3.2.2. Freeze drying

Various studies have shown that freeze drying is a favoured method for increasing the shelf life of foods. Drying is applied at very low temperatures (e.g. – 45 C°) and the drying medium is vacuumed and thus free of air. This air free drying medium prevents oxidation and chemical change of product (Longmore, 1971). This process is slow and expensive when compared to other drying methods where heat is used. It is especially used in high value added products to protect the quality.

Working mechanism of freeze drying is based on dehydration by sublimation of the ice fraction of a frozen product. Freezing, primary drying (sublimation) and secondary drying (desorption) are the three steps of freeze drying (Barbosa-Canovas and Vega-Mercado, 1996; Ratti, 2008; Tang and Pikal, 2004).

Firstly freezing of the liquid sample takes place. During freezing, as liquid is chilled, water portion of liquid forms ice crystals. As the freezing proceeds, frozen part of the liquid becomes bigger. Concentration of the remaining liquid increases because amount of water in the liquid decreases. As water content of liquid decreases, liquid becomes more concentrated and as a result of this viscosity of the liquid suspension increases. Finally liquid solidifies completely and crystalline structure is formed (Franks, 1990).

After freezing, primary drying stage comes. At this stage, ice portion of the product sublimates. In secondary drying, remaining water in the sample is removed from the product(Pikal et al., 1990).

Microencapsulation of different types of oil by freeze drying was studied by various researchers. Microencapsulated fish oil production by freezing and subsequent freeze drying yields high-quality products (Heinzelmann and Franke, 1999). In their study, Koc et al. (2010) used gelatin, pullulan, lactose and sucrose as coating material for microencapsulation of fish oil by freeze drying and they found that using these wall materials are suitable for preparation of freeze-dried fish oil microcapsules in order to obtain more stable fish oil against oxidation. Quispe et al. (2011) investigated microencapsulation of flax oil with zein using spray and freeze drying separately. The maximum microencapsulation efficiencies were found as 93.26% and 59.63 % for spray drying and freeze drying, respectively. Microcapsules prepared by freeze drying resulted in agglomerated small spheres.

Desobry et al. (1997) compared spray drying, drum drying and freeze drying for encapsulation of β-carotene. They found that drying and encapsulation process led to an 8% degradation of β-carotene with freeze drying, 11% with spray drying and 14% with drum drying. It was also found that the drum-dried product provided the best encapsulation with only 24% surface carotene as compared to the spray dried (38% surface carotene) and freeze-dried (35% surface carotene) ones.

1.3.3. Materials used for encapsulation

Choosing suitable wall material is important since it will affect characteristics of emulsion, retention of volatiles in the case of flavour encapsulation and stability of the final product. Mixtures of different wall materials are used to achieve desirable properties because one material generally doesn't meet all. For instance a mixture of maltodextrin and gum arabic was reported to be an effective wall material for encapsulation of soy oil (McNamee et al., 2001) and fatty acids (Teixeira et al., 2004; Minemoto et al., 2002).

Only aqueous-based dispersions are used in the food industry. Thus the wall material requires good solubility in water. A good wall material should have high solids at low viscosity in emulsion, good film forming ability and emulsifying properties.

Ratio of core material to wall material effects surface oil amount in encapsulated product for oil encapsulation. For example increasing oil loads generally lead to lower encapsulation efficiency (Hogan et al., 2001; Bertolini et al., 2001; Tan et al., 2005). In their study, Hogan et al. (2001) found that when soy oil-sodium caseinate ratio increased from 0.25 to 3.0, microencapsulation efficiency was steeply decreased from about 90% to 20% respectively in the spray drying period.

For volatile materials and oil encapsulation in particular, the ideal wall material should have emulsifying properties; have good taste; have film forming ability; have low viscosity at high solids levels; stable in storage; have low hygroscopicity; release the flavour when dissolved in water in the final food product; be economical and provide good protection against environmental factors (Desai and Park, 2005; Bangs, 1985; Brazel, 1999) . Mixtures of different wall materials are used in order to provide those required properties. Wall materials generally used for encapsulation process includes a group of carbohydrates which are modified starches, maltodextrin, cellulose derivatives and different gum types. Protein group used as wall material includes gluten, whey protein, caseinate and gelatin. Maltodextrin and whey protein concentrate (WPC) used as coating material in this study.

1.3.3.1. Whey Proteins

Whey protein is derived from whey which is separated as liquid phase from coagulum during cheese making. Major components of whey protein includes alpha-lactalbumin, beta-lactoglobulin(about 70%) and also it contains glycomacropeptide and serum albumin.

Solubility of whey proteins depends on ionic charge and pH and temperature of the medium. Whey proteins are globular proteins and they are soluble in the milk (Chen et al., 2006). At their isoelectric point (about pH 5) whey protein becomes insoluble and it starts to aggregate. Denaturation of whey protein at temperatures above 75°C occurs and because of denaturation it becomes insoluble. Globular proteins have both hydrophilic and hydrophobic parts in their

structure because of that they have emulsifying property. In an emulsion containing oil, water and globular protein, protein forms a continuous layer around oil droplets (Lefevre and Subirade, 2003; Chen et al., 2006). Protein films around droplets provide charged layers and this layers prevent flocculation and coalescence. Whey protein was reported to be an effective wall material for encapsulation of volatile esters (Sheu and Rosenberg, 1995; Rosenberg and Sheu, 1996) and anhydrous milk fat (Young et al., 1993; Moreau and Rosenberg, 1996). When whey protein isolate used as a wall material, it was found that it protected orange oil against oxidation effectively (Kim and Morr, 1996). According to results of another study, using whey protein concentrate (WPC) as a wall material for encapsulation of soybean oil led to higher surface oil content when compared to sodium caseinate (Fäldt and Bergenståhl, 1996). Hogan et al., (2001) studied microencapsulating properties of whey protein concentrate. Results of the study showed that for the oil/protein loads examined, whey protein concentrate provides surface active properties for encapsulation of soy oil however it has limited ability for providing good redispersion and for maintaining stability during spray drying.

1.3.3.2. Maltodextrins

Maltodextrin (MD) is an oligosaccharide that is produced by partial hydrolysis of starch. Hydrolysis is usually catalysed by an acid or enzyme. It has hygroscopic property. It is almost tasteless and easily digestible. Maltodextrins have low viscosity at high solids content which is a desirable property for wall material used in encapsulation.

Maltodextrins provide structural integrity to the final product.The dextrose equivalent value of maltodextrins which is used to indicate the degree of hydrolysis of starch into glucose syrup has been used for the selection of maltodextrins in applications (Kenyon and Anderson, 1988). Encapsulated powders having high dextrose equivalent value have high encapsulation efficiencies and are less permeable to oxygen. (Sheu and Rosenberg, 1998; Re, 1998; Hogan et al., 2003). However, maltodextrin is lacking in emulsifying properties and according to some studies results in poor retention of flavors (Bangs, 1985; Bangs and Reineccius, 1990). Therefore, it is desirable to blend MD with other ingredients having good emulsifying properties such as gum Arabic, (Bhandari et al., 1992; Liu et al., 2000; Sankarikutty et al., 1988) or milk proteins (Kagami et al., 2003; Hogan et al., 2003).

In their work, microencapsulation of ginger essential oil in MD/whey protein isolate (WPI), Toure et al. (2007) investigated the effect of wall composition on surface oil content and oxidation of oil. They found that MD/ WPI ratio of 1:1 and core to wall ratio of 1:4 produced the lowest surface oil (0.07 g/100 g) and improved stability during storage. Bylaite et al. (2001) investigated the properties of caraway essential oil encapsulated only with WPC and with mixture of WPC and MD. When mixture of WPC and MD was used as wall material, it was found that retention of volatiles increased during spray drying and also protective property of wall material against oxidation increased in encapsulated powder.

1.4. Objective of the Study

Wheat germ oil contains high amount of vitamin E and polyunsaturated fatty acids mainly linoleic acid and linolenic acid (Wang and Jonson, 2001; Megahad and El Kinawy, 2002). Therefore, consumption of wheat germ oil is very beneficial for health. However, high content of polyunsaturated fatty acids makes the oil highly prone to oxidation. Therefore, there are difficulties in storage and incorporation of wheat germ oil in foods in its free form. Oxidation problem can be overcome and storage life can be extended by means of encapsulation. In addition, encapsulated oil can be added to foods in order to increase nutritive value. Thus, functional foods containing wheat germ oil can be developed.

However, there is lack of study in literature in encapsulation of wheat germ oil. The objective of this study is to determine the best encapsulation conditions of wheat germ oil. The effects of different core to coating ratios, maltodextrin and whey protein concentrate ratios and ultrasonication time on the encapsulation efficiency, particle size distribution and surface morphology of the encapsulated powder were also studied.

CHAPTER 2

MATERIALS & METHODS

2.1. Materials

Whey protein concentrate (WPC) was purchased from Tunçkaya Kimyevi Maddeler (Tuzla, İstanbul). Maltodextrin (MD) having dextrose equivalent (DE) value of 4.5-8.5 (C*Dry MD 01955) was obtained from Cargill Foods (Istanbul, Turkey) and wheat germ oil was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Hexane, hydrochloric acid, sodium hydroxide, dipotassium phosphate and potassium dihydrogen phosphate were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck Chemical Companies (Deisenhofen, Germany). They were analytical grade.

2.2. Encapsulation process

Encapsulation process was mainly composed of five main steps which were coating material preparation, coarse emulsion preparation, ultrasonication, freezing and freeze drying of emulsion and finally milling of dried samples.

2.2.1. Preparation of coating material solution

Hydrated solutions of MD at different concentrations (10%, 20%, 30% by weight) were prepared by mixing maltodextrin with distilled water using magnetic stirrer (Heidolph MR 3001 K, Heidolph Instruments GmbH & Co, Schwabach, Germany). In the case of WPC, their solutions were prepared by dispersing the necessary amount of their powder into buffer solution (5 mM phosphate buffer, pH 7) to obtain different concentrations (10 %, 20 %, 30 % by weight) using magnetic stirrer. Mixing continued until the materials were dissolved. Then, pH of WPC solution was adjusted to pH 7.0 using 1 M HCl and 1 M NaOH if required.

For the preparation of phosphate buffer, first, 1M solutions of dipotassium phosphate (K_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) were prepared separately. After that for obtaining 100 ml pH 7 phosphate buffer; 61.5 ml K_2HPO_4 and 38.5 ml KH_2PO_4 were mixed. In order to get 5mM phosphate buffer dilution was made (Kuhlmann, 2006).

MD and WPC solutions were prepared one day before the emulsification process and kept for one night in a shaking water bath (GFL 1086, Burgwedel, Germany) at 25 C° and 90 rpm.

2.2.2. Coarse emulsion preparation

In order to get a stable emulsion with ultrasound, coarse emulsion preparation is a crucial step. Therefore, after preparation of coating material solutions separately, a pre-emulsion must be formed by mixing equal amounts (by weight) of maltodextrin and whey protein concentrate solutions at the required concentrations to obtain total solids content of 40 % (w/w) and MD:WPC ratios of 1:3, 2:2 and 3:1. Wheat germ oil is then added to obtain the desired core to coating ratios of 1:8 and 1:4 at 5000 rpm and for 5 min using high-speed blender (IKA T25 digital Ultra-Turrax, Selangor, Malaysia). Mixing was performed in 250 ml beaker for 100 g solution.

2.2.3. Ultrasonication

Ultrasonic Homogenizer (Sonic Ruptor 400, OMNI International the Homogenizer Company, GA, USA) was used for emulsification. Ultrasonic homogenizer was equipped with titanium probe with diameter 25.4 mm. Line voltage is transformed to electrical energy by ultrasonic power supply. Probe of ultrasound converts this electrical energy into mechanical energy.Titanium tip is the part that intensifies vibrations transmitted from probe. As probe vibrates , it transfers this motion to the titanium tip submerged to the solution. Ultrasonic homogenization was performed using % 80 power and different times (2 min, 5 min, 10 min). During ultrasonication as some of the energy was dissipated into heat, in order to control temperature rise, outer surface of the beaker was covered with ice bag. Every batch was done in 250 ml beaker that contains 100 g of solution.

2.2.4. Freezing and freeze drying

Immediately after emulsification with ultrasound, samples were transferred to 100 ml beakers half fully and placed into freezer at about -4 C°. Frozen samples were then dried under vacuum at -50 C° and 0.007 atm for 48 h.

2.2.5. Grinding of dried samples

After freeze drying process, sample was grinded by using a glass rod in order to transform it to powder form.

2.3. Particle size analysis of emulsions

Laser light scattering method was used for particle size anlaysis. Mastersizer 2000 (Malvern Instruments Limited, Worcestershire, UK) was used as equipment. Particle refractive index value was 1.52 and dispersant refractive index value was 1.33. The absorption index was 0.1 for equipment during the measurement.
The particle size was described as surface-weighted mean diameter, D[3,2]. It was calculated according to equation (2). Width of the particle size distribution was defined by span and it was calculated from equation (3).

$$D_{32} = \frac{\sum n_i \, d_i^3}{\sum n_i \, d_i^2} \quad (2)$$

$$Span = \frac{[d(v,90) - d(v,10)]}{d(v,50)} \quad (3)$$

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

2.4. Analysis of encapsulated powder

2.4.1. Encapsulation efficiency analysis

Encapsulation efficiency analysis was adapted from Millqvist-Fureby (2003). 5 gram of powder was mixed with 50 ml n-hexane and shaken at the magnetic stirrer at 200 rpm for 60 sec. This suspension was allowed to stand for 10 min and then filtered through filter paper (No. 41, Whatman, Maidstone, UK). Then, the powder residue was washed with 2×5 ml hexane. Extracted oil was transferred to a beaker and solvent was evaporated under the fume hood. After evaporation of solvent, beaker was dried in an oven at 105°C until constant weight (about 1 h) was reached. Weight of beaker containing extracted oil residue was subtracted from weight of initial clean and dry beaker and thus extracted oil amount was calculated. Encapsulation efficiency was calculated by the following equation:

$$EE(\%) = \frac{TO - SO}{TO} \times 100 \quad (1)$$

Where, EE is the encapsulation efficiency, TO is the total amount of oil present in the encapsulated powder and SO is the amount of oil on the surface of the capsules, which is extracted using the method mentioned above.

2.4.2. Surface morphology of capsules

Scanning electron microscope was used to investigate the surface morphology and the microstructural properties of the freeze dried encapsulated powders. Samples were coated with a very thin layer of a gold-palladium (Au-Pd) alloy using HUMMLE VII Sputter Coating Device (ANATECH, Union city, CA, USA). Coated samples were analysed using scanning electron microscopy (SEM) (JSM-6400 Electron Microscope, Jeol Ltd., Tokyo, Japan) equipped with NORAN System 6 X-ray Microanalysis System and Semafore Digitizer and operating at two different voltages which were 10 kV and 20 kV. Images were taken at ×50 magnification. The images of the encapsulated powders at microscopic scale were taken by the equipment's software installed on a computer connected to the system.

2.5. Statistical Analysis

The effects of MD:WPC ratio, core to coating ratio, and ultrasonication time on encapsulation efficiency and particle size were determined by using analysis of variance (ANOVA) using SAS software version 9.1 (SAS Institute Inc., NC, USA). If results were significantly different than each other , Duncan's Multiple comparison test was used for comparisons ($p \leq 0.05$). All the results were the average of two replication

CHAPTER 3

RESULTS AND DISCUSSION

In the first part of the study, five different core to coating ratios were tried which were 1:8, 1:4, 2:4, 3:4, 4:4. As coating material maltodextrin (MD) and whey protein concentrate (WPC) mixture, at different ratios, which were MD:3-WPC:1, MD:2-WPC:2; MD:1-WPC:3; were used. For these experiments ultrasound power was 80% and ultrasonication time was 2 min. After selecting the samples that had higher encapsulation efficiency, the effects of ultrasonication time (2 min, 5 min and 10 min) on encapsulation effciency, particle size distribution and surface morphology were also studied in the second part of the study.

3.1. Encapsulation efficiency of encapsulated powder

According to Table 3.1; it can be seen that as core to coating ratio increased surface oil content increased at constant MD:WPC ratios which implied that encapsulation efficiency decreased. It can be said that as the amount of oil increases, encapsulation becomes more difficult and more surface oil exists on encapsulated powder and thus encapsulation efficiency decreases. As can be seen in the Figures 3.1-3.3, 1:8 core to coating ratio had the highest encapsulation efficiency values (53.8%, 65.35% 79.2% , for MD:WPC ratio of 3:1, 2:2 and 1:3, respectively). From these results it can be inferred that surface oil of the encapsulated powder decreased as oil amount decreased. In addition, increase in the concentration of WPC, increased encapsulation efficiency. Encapsulation efficiency was quite satisfactory also when core to coating ratio was 1:4 and MD:WPC ratio was 1:3 (74.35%). Coating formulation that had higher WPC had better encapsulating property which can be due to good emulsifying property of WPC. Similarly, Jimenez et al. (2006) found that the surface oil contents of microcapsules prepared with WPC-MD and gum arabic (GA) were higher than those prepared with WPC only. This result showed that WPC provided better encapsulation efficiency values compared to WPC-MD and GA. Vanzo et al. (2008) used MD and whey protein isolate as wall constituents in different proportions for microencapsulation of coffee oil and showed that higher values of encapsulation efficiency were obtained when carrier solutions with higher concentration of protein were used. These results were in agreement with our findings.

In the second part of the study, among fifteen different combinations of MD and WPC and core to coating ratios, four of them which had the highest encapsulation efficiency values were chosen and the effects of longer ultrasonication time on encapsulation efficiency were studied.

Table 3.1 Surface oil content (g/g) of capsules prepared with different maltodextrin (MD): whey protein concentrate (WPC) ratios in the coating formulation and different core to coating ratios when 2 min ultrasonication was used in preparation of emulsions.

MD:WPC	Core:coating	Surface oil content (g/g)
3:1	1:8	0.20
3:1	1:4	0.37
3:1	2:4	0.77
3:1	3:4	1.23
3:1	4:4	1.51
2:2	1:8	0.15
2:2	1:4	0.45
2:2	2:4	0.96
2:2	3:4	1.30
2:2	4:4	1.59
1:3	1:8	0.09
1:3	1:4	0.20
1:3	2:4	0.80
1:3	3:4	1.28
1:3	4:4	1.56

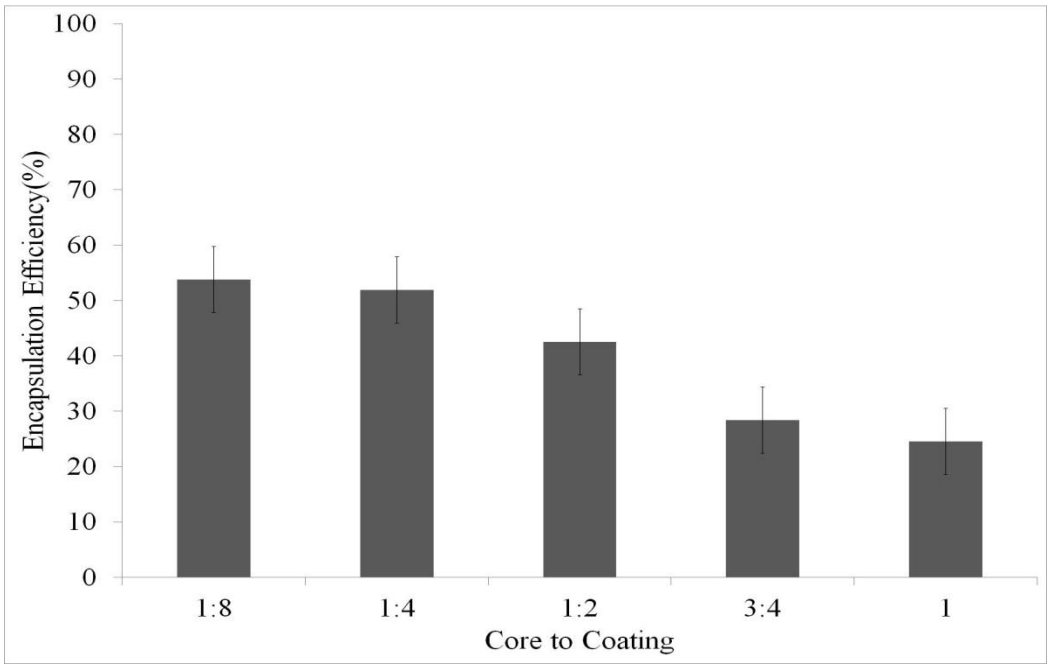

Figure 3.1 Variation of encapsulation efficiency as a function of core:coating ratio when MD:WPC was 3:1 in the coating formulation and for 2 min US time

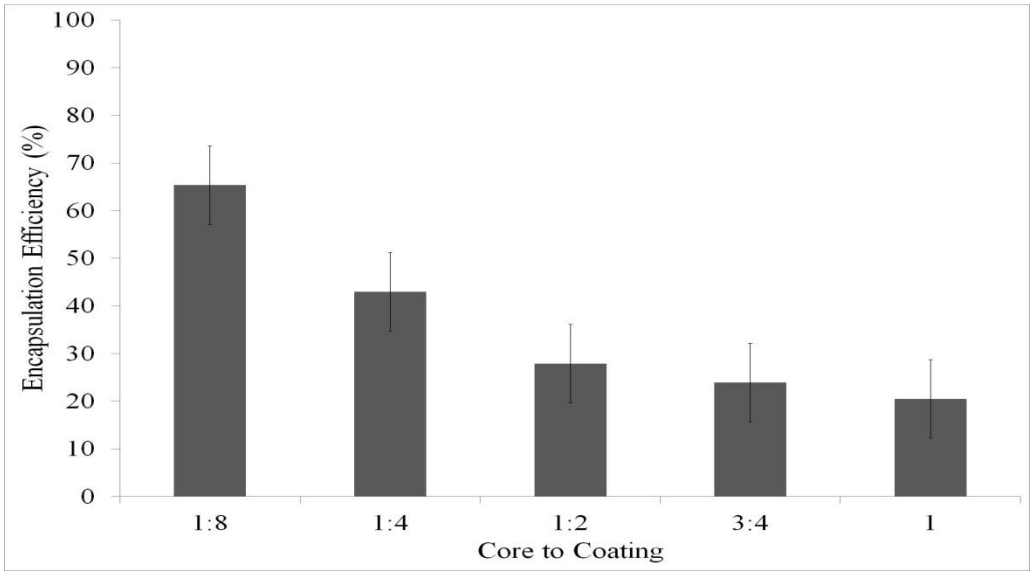

Figure 3.2 Variation of encapsulation efficiency as a function of core:coating ratio when MD:WPC was 2:2 in the coating formulation and for 2 min US time

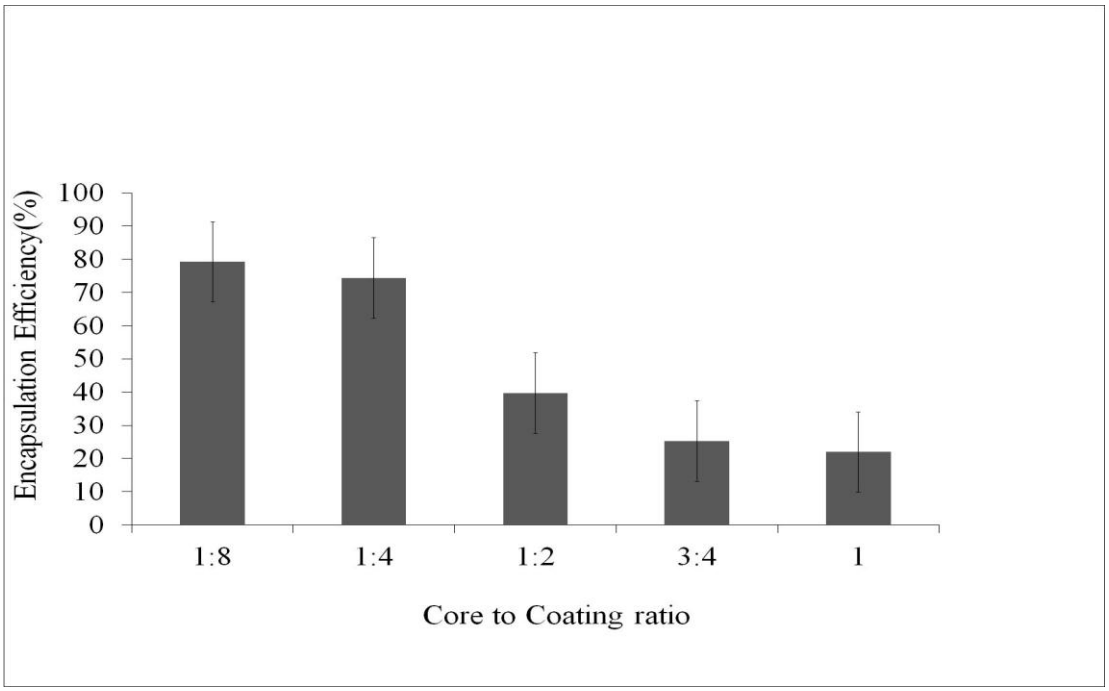

Figure 3.3 Variation of encapsulation efficiency as a function of core:coating ratio when MD:WPC was 1:3 in the coating formulation for 2 min US time

In general, increasing ultrasonication time from 2 min up to 10 min had a positive effect on encapsulation efficiency and it was seen that the effect of ultrasonication time was dependent on the composition of powders (Table 3.2).

According to one way ANOVA, it was seen that for MD:WPC ratio of 3:1 and core to coating ratio of 1:8, 2 min and 5 min ultrasound applications were not significantly different but 10 min provided higher encapsulation efficiency value (Table 3.2, Appendix Table A1). Cilek et al. (2012) studied microencapsulation of phenolic compounds by using ultrasonication and found that samples prepared by 5 and 10 min ultrasonication had lower encapsulation efficiency as compared to samples prepared by 15, 20 and 25 min of ultrasonication.

In the case of MD:WPC ratio of 2:2, core to coating ratio of 1:8 and MD:WPC ratio of 1:3 and core to coating ratio of 1:4, 2 min and 10 min ultrasonication times were significantly different from each other with respect to their effect on encapsulation efficiency. On the other hand, ultrasonication time had no significant effect on encapsulation efficiency when coating with MD:WPC ratio of 1:3 and core to coating ratio of 1:8 was used in encapsulation.

Table 3.2 Effect of ultrasonication time on encapsulation efficiency values

MD:WPC	Core:Coating	Ultrasonication time (min)	Encapsulation efficiency (%)
3:1	1:8	2	53.80 ^{e*}
3:1	1:8	5	51.29 ^{e}
3:1	1:8	10	75.10 ^{bcd}
2:2	1:8	2	65.35 ^{d}
2:2	1:8	5	70.30 ^{cd}
2:2	1:8	10	81.59 ^{abc}
1:3	1:8	2	79.20 ^{abc}
1:3	1:8	5	85.56 ^{ab}
1:3	1:8	10	85.37 ^{ab}
1:3	1:4	2	74.35 ^{bcd}
1:3	1:4	5	77.54 ^{bc}
1:3	1:4	10	89.62 ^{a}

*Encapsulation efficiency values having different superscript letters are significantly different ($p \leq 0.05$).

Increasing concentration of WPC in coating, increased encapsulation efficiency when 2 min or 5 min ultrasonication was used (Table 3.2). However, increasing the amount of WPC in coating formulation had no significant effect on encapsulation efficiency when 10 min ultrasonication was applied.

3.2. Particle size analysis of encapsulated powder

Experimental data for particle size analysis were given in Appendix B. Particle size distribution of encapsulated powders were compared in terms of Sauter mean diameter (D[3,2]) and span. Effect of different ultrasonication times on particle size can be analysed when the same coating formulation and core to coating ratios are compared. For MD:WPC ratio of 3:1 and core:coating ratio of 1:8 mean particle size expressed as Sauter mean diameter (D[3,2]) was larger for 2 min ultrasound application although there was no significant difference between 5 min and 10 min ultrasonication (Table 3.3, Appendix Table A.2). In the case of MD:WPC ratio of 2:2 and core:coating ratio of 1:8, the same trend was observed. For MD:WPC ratio of 1:3, in the case of core:coating ratio of both 1:4 and 1:8, there was significant difference between D[3,2] values of encapsulated products for different ultrasonication times and as time increased mean particle size decreased. In Figure 3.4, difference between particle size distribution of encapsulated powders for

different ultrasonication times can be clearly seen for MD:WPC ratio of 1:3 and core:coating ratio of 1:4. As ultrasonication time increased, the particle size distribution curve shifted to the left meaning that microcapsules with smaller particle size were obtained. This is similar to the results found in literature. In the study of Jafari et al. (2007) sonication time was found to have a significant effect ($P<0.05$) on D[3,2] up to 60s, that is as sonication time increased D[3,2] decreased. Kentish et al. (2007) applied 200 W ultrasonic power to emulsion of flaxseed oil, water and Tween 40 and as sonication time increased from 1 min to 3 min, D[3,2] of emulsion decreased from 0.35 microns to 0.15 microns. Cucheval and Chow (2008) used ultrasound for preparation of emulsion made of soybean oil, water and tween 80 and found that droplet size D[4,3] decreased sharply between 0-3 min. Abismail et al. (1999) showed that D[3,2] of oil-in-water emulsion which consists of water/kerosene (oil)/polyethoxylated sorbitan monostearate (surfactant) decreased with sonication time from 5 s to 30 s.

Table 3.3 Particle size analysis results of emulsions

MD:WPC	Core:Coating	Ultrasonication time (min)	Particle size (D[3,2]) (μm)	SSA * (m^2/g)	Span
3:1	1:8	2	2.43 [a]	2.46	1.63
3:1	1:8	5	2.02[cb]	2.96	1.47
3:1	1:8	10	2.01[cb]	2.98	1.27
2:2	1:8	2	2.15 [b]	2.79	1.59
2:2	1:8	5	1.82[dc]	3.28	1.71
2:2	1:8	10	1.68[d]	1.50	3.57
1:3	1:8	2	1.60[d]	3.57	4.26
1:3	1:8	5	1.21[e]	4.95	21.07
1:3	1:8	10	0.41[f]	15.59	18.60
1:3	1:4	2	1.68[d]	3.75	1.60
1:3	1:4	5	1.00[e]	4.75	24.06
1:3	1:4	10	0.40 [f]	52.87	11.70

*SSA: specific surface area

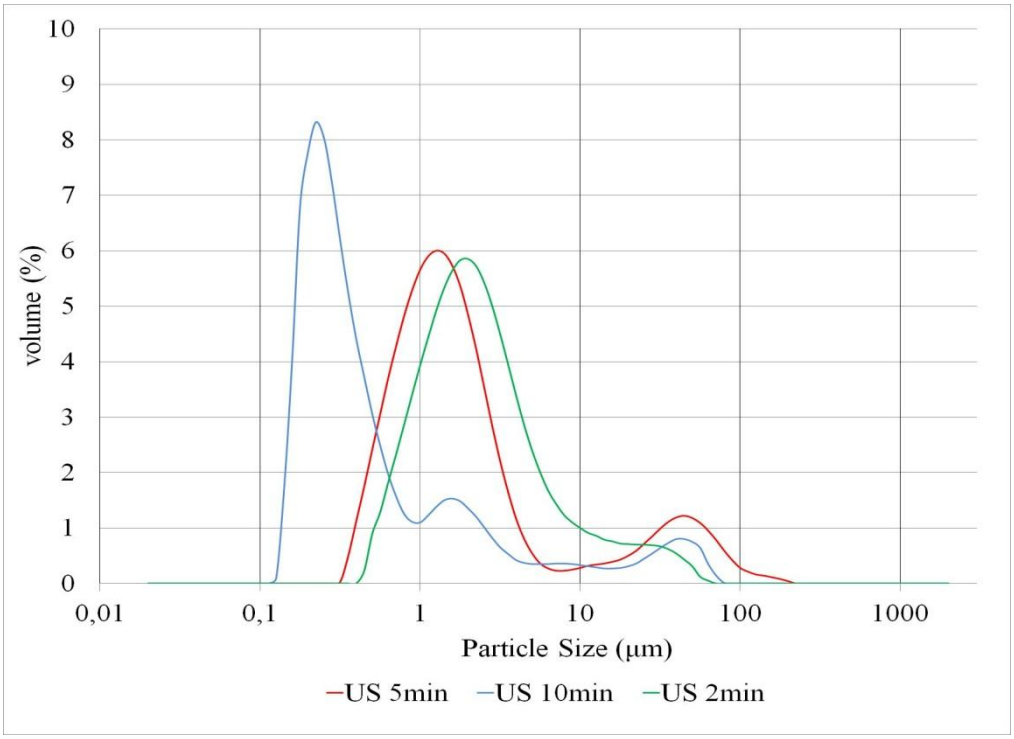

Figure 3.4 Particle size distribution of emulsions having MD:WPC ratio of 1:3 and core to coating ratio of 1:4

As WPC concentration increased in the coating formulation, the mean diameter of the particles decreased significantly (Table 3.3). This can also be seen in Figure 3.5, in which particle size distribution curve shifted to the left (smaller size). This could be explained by the good emulsifying property of WPC related to the many hydrophobic and hydrophilic parts it has. More amount of WPC could lead to better emulsification and oil globules could disperse finely and as a result of this particle size of emulsion could be smaller. It was also observed that as WPC concentration and ultrasonication time increased, specific surface area increased (Table 3.3).

On the other hand, increasing WPC in the coating, increased span of the particle size distribution significantly especially for 5 min and 10 min ultrasonication times (Figure 3.5). It can also be seen from Table 3.3 that for 5 and 10 min ultrasonication, span of the samples which had coating ratio of MD:1 WPC:3 were wider when compared to MD:3 WPC:1 and MD:2 WPC:2 ratios. Wider span of samples could be explained by higher amount of WPC in the coating. During ultrasonication, some amount of energy given to the system is dissipated as heat which increases the temperature of the sample. WPC could denature and form aggregates at higher temperatures (above 60°C). These aggregates have larger particle size and thus sample contains both smaller and larger particles in it. Wider distribution of particle size could be due to this reason.

Increasing the amount of oil as compared to its coating did not have any significant effect on mean particle size. According to Table 3.3 for the same ultrasonication time (that is valid for 2, 5 and 10 min application), Sauter mean diameter (D[3,2]) of samples having MD:1-WPC:3 coating formulation but different core to coating ratioswere not significantly different.

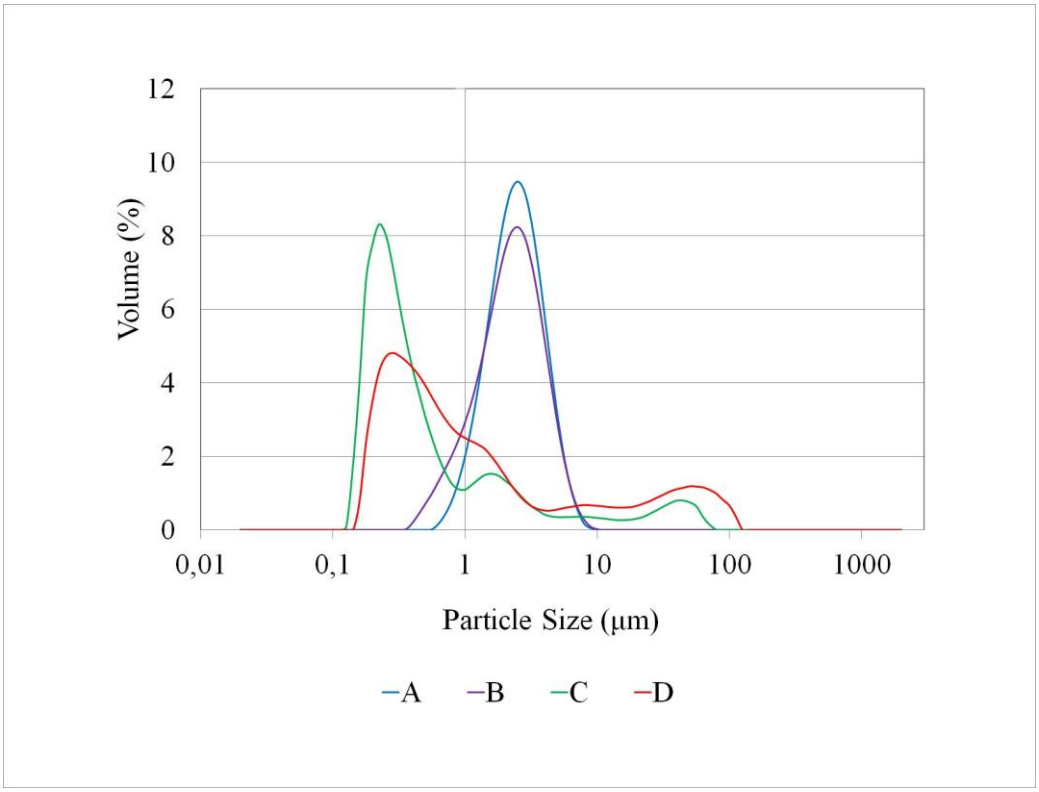

Figure 3.5 Particle size distribution of emulsions having different MD:WPC and core:coating ratios for 10 min sonication; A: MD:3 WPC:1 Oil: 0,5; B: MD:2 WPC:2 Oil:0,5;
C: MD:1WPC:3 Oil:1; D: MD:1 WPC:3 Oil: 0,5

3.3. Surface morphology of encapsulated powder

In this part of the study, it was aimed to observe outer surface properties of encapsulated powders and to check differences between encapsulated powders that were produced under different conditions.

It is known that freeze dried powder has generally irregular shape, very light, highly porous structure as compared to other drying techniques such as spray drying (Anwar and Kunz, 2011). It is seen that freeze dried powders have larger surface area Our SEM results are in accordance with these properties, additionally they had slab like shapes, some of them had layer by layer structure.

In Figure 3.6, it can be seen that particle size of samples that were treated with 5 and 10 min ultrasonication was smaller than samples prepared with 2 min ultrasonication for MD:2 WPC:2 and

core to coating ratio of 1:8. This result is supported also by our particle size values (Table 3.3). As ultrasonication time increased from 2 min to 5 min, particle size was decreased but there was no significant difference between 5 and 10 min application.

In Figure 3.7, it can be seen that for the same time of ultrasonication, the surface of powder having higher concentration of WPC (Figure 3.7C) was smoother whereas surface of powder that had higher MD concentration (Figure 3.7A) was rougher. In literature, there are some studies which show that the structures of microcapsules are affected by the ratio of coating formulation. Sheu and Rosenberg (1998) reported that when whey protein isolate:maltodextrin ratio was increased from 1:19 to 3:1 this caused encapsulated powder surface become smoother and surface cracks were decreased. Moreover, Jafari et al. (2007) observed that addition of WPC into emulsion composition had changed the structure and surface morphology of the encapsulated oil by decreasing surface dents and increasing smoothness. They suggested that this could be due to drying of wall matrix at a slower rate with WPC samples, and probably WPC provides elasticity to wall systems. The results observed from SEM images can be related to encapsulation efficiency. Smoother surface of microcapsules prepared with higher WPC concentration provided lower surface oil content (Table 3.1) and higher encapsulation efficiency (Figure 3.1-3.3). Sheu and Rosenberg (1998) found that at a WPI:MD ratio of 1:9 or 1:1, microcapsules had fewer surface dents than those at a 1:19 ratio. Surface indentation can be correlated with extractable surface oil amount. When surface oil is extracted from capsules having more dents, more oil would be extracted because solvent will reach inner surfaces, extract more oil and this will decrease encapsulation efficiency. When different core to coating ratios are compared at the same ultrasonication time (Figure 3.8) it was seen that there was no significant difference between SEM images.

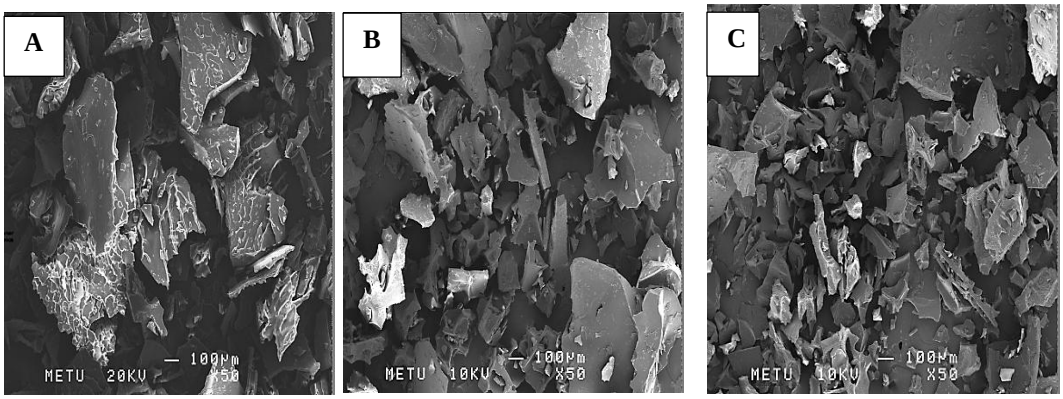

Figure 3.6 SEM images of microcapsules prepared with core to coating ratio of 1:8, MD:2 WPC:2 coating ratio and different ultrasonication times with x50 magnification (A): 2 min (B): 5 min (C): 10 min

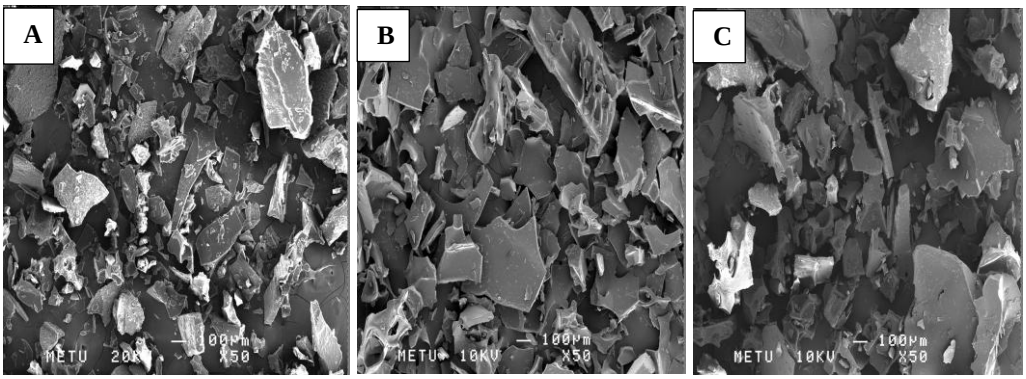

Figure 3.7 SEM images of microcapsules prepared with the same time of ultrasonication time (5 min), same core to coating ratio (1:8) and different coating ratios (A) MD:3 WPC:1 (B) MD:2 WPC:2 (C) MD:1 WPC:3 (x50 magnification)

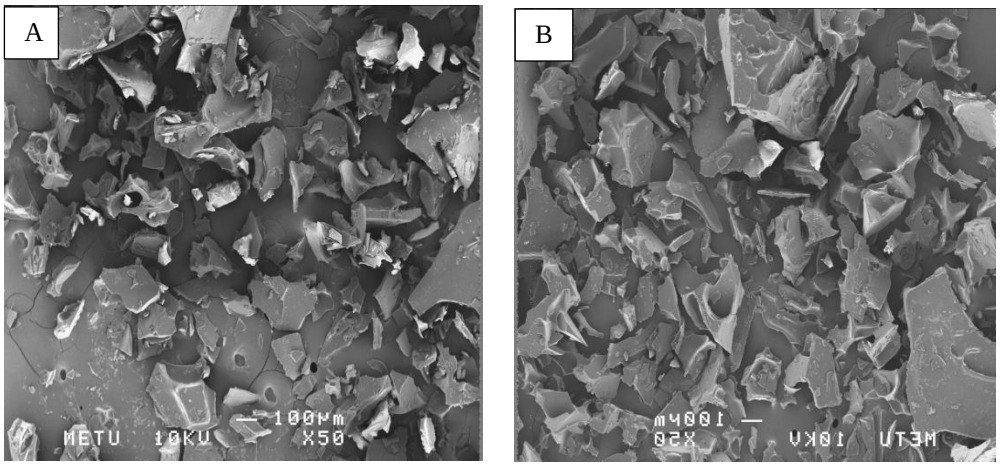

Figure 3.8 SEM images of microcapsules prepared with same time of ultrasonication (10 min) with a coating ratio of MD:1 WPC:3 and different core to coating ratios (A) Core to coating 1:4 (B) Core to coating 1:8

CHAPTER 4

CONCLUSION & RECOMMENDATIONS

In this study, wheat germ oil was encapsulated by using ultrasonic treatment and freeze drier and maltodextrin and whey protein concentrate were used in different ratios as a coating material.

Among five core to coating ratios, 1:8 gave the best result in terms of encapsulation efficiency. As a common trend, it was observed that increasing WPC ratio in coating resulted in both higher encapsulation efficiency and smaller particle sizes. Increasing ultrasonication time also had a positive effect on encapsulation efficiency.

As a conclusion, microcapsules that were coated with MD:WPC ratio of 1:3 with core to coating ratio of 1:8 and prepared by ultrasonication for 10 min can be utilized as a functional food because this formulation had the highest encapsulation efficiency and the smallest particle size.

As a recommendation, further study can be done for determining the oxidative stability of encapsulated powder during storage at different relative humidity and temperatures for a certain time period. Incorporation of encapsulated powder to foods such as cake or bread can also be studied and thermal stability and bioavalibility of those functional foods can be analysed. Additionally, further studies can be done for obtaining microcapsules that have both high encapsulation efficiency and high oil load at the same time. This is important because higher oil load in microcapsules enables to utilize more oil for certain amount of encapsulated powder.

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APPENDIX A

STATISTICAL ANALYSES

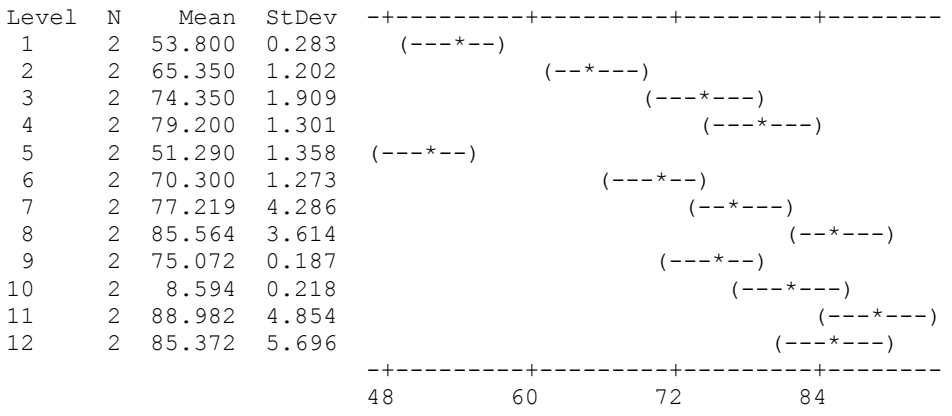
Table A.1 Effect of ultrasonication time on encapsulation efficiency values

One-way ANOVA: response versus Treatment

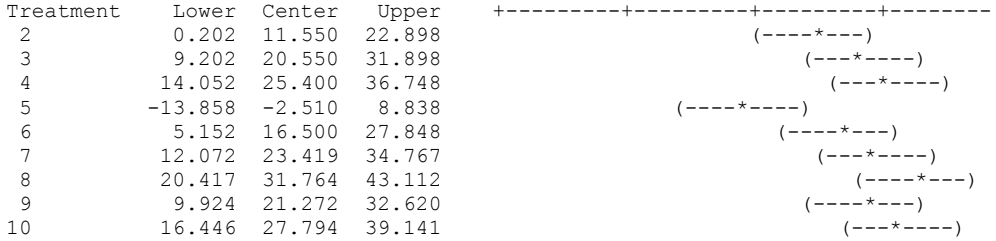
Source	DF	SS	MS	F	P
Treatment	11	3192.34	290.21	35.59	0.000
Error	12	97.85	8.15		
Total	23	3290.19			

S = 2.855 R-Sq = 97.03% R-Sq(adj) = 94.30%

Individual 95% CIs For Mean Based on Pooled StDev



Pooled StDev = 2,855
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatmen
Individual confidence level = 99,82%



```
11          23.835  35.182  46.530                                  (---*---)
12          20.225  31.572  42.920                                (----*---)
                                    +---------+---------+---------+---------
                                   -50       -25         0        25
```

Treatment = 2 subtracted from:

```
Treatment   Lower   Center   Upper    +---------+---------+---------+---------
 3          -2.348    9.000  20.348                       (----*---)
 4           2.502   13.850  25.198                         (----*---)
 5         -25.408  -14.060  -2.712               (---*----)
 6          -6.398    4.950  16.298                      (----*----)
 7           0.522   11.869  23.217                         (----*---)
 8           8.867   20.215  31.562                             (---*----)
 9          -1.626    9.722  21.070                       (----*---)
10           4.896   16.244  27.591                          (---*----)
11          12.285   23.632  34.980                              (---*----)
12           8.675   20.022  31.370                           (----*----)
                                  +---------+---------+---------+---------
                                 -50       -25         0        25
```

Treatment = 3 subtracted from:

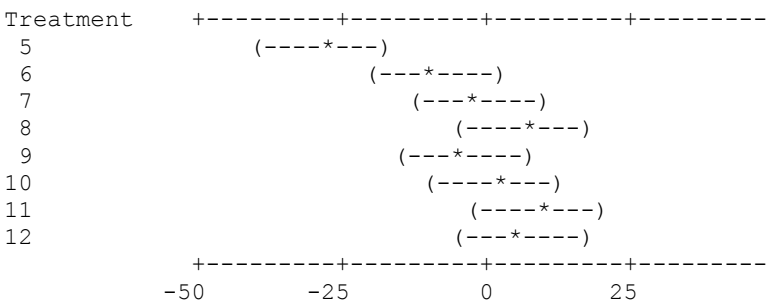
```
Treatment    Lower   Center    Upper
 4          -6.498    4.850   16.198
 5         -34.408  -23.060  -11.712
 6         -15.398   -4.050    7.298
 7          -8.478    2.869   14.217
 8          -0.133   11.215   22.562
 9         -10.626    0.722   12.070
10          -4.104    7.244   18.591
11           3.285   14.632   25.980
12          -0.325   11.022   22.370
```

```
Treatment    +---------+---------+---------+---------
 4                        (----*---)
 5              (----*---)
 6                      (---*----)
 7                         (---*----)
 8                            (---*----)
 9                          (---*----)
10                            (----*---)
11                               (----*---)
12                            (---*----)
             +---------+---------+---------+---------
            -50       -25         0        25
```

Treatment = 4 subtracted from:

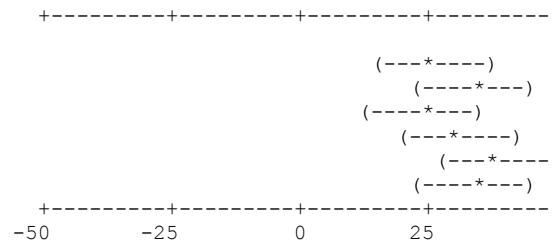
```
Treatment    Lower   Center    Upper
 5         -39.258  -27.910  -16.562
 6         -20.248   -8.900    2.448
```

```
 7         -13.328   -1.981    9.367
 8          -4.983    6.364   17.712
 9         -15.476   -4.128    7.220
10          -8.954    2.394   13.741
11          -1.565    9.782   21.130
12          -5.175    6.172   17.520
```



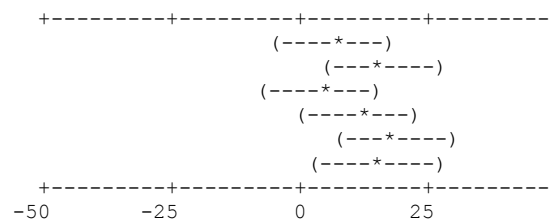
Treatment = 5 subtracted from:

```
Treatment   Lower  Center   Upper
 6          7.662  19.010  30.358
 7         14.582  25.929  37.277
 8         22.927  34.274  45.622
 9         12.434  23.782  35.130
10         18.956  30.304  41.651
11         26.345  37.692  49.040
12         22.735  34.082  45.430
```



Treatment = 6 subtracted from:

```
Treatment   Lower  Center   Upper
 7         -4.428   6.919  18.267
 8          3.917  15.264  26.612
 9         -6.576   4.772  16.120
10         -0.054  11.294  22.641
11          7.335  18.682  30.030
12          3.725  15.072  26.420
```



Treatment = 7 subtracted from:

```
Treatment   Lower  Center   Upper
 8          -3.002   8.345  19.693
 9         -13.495  -2.147   9.200
```

```
10          -6.973   4.375  15.722                    (----*---)
11           0.415  11.763  23.111                       (----*---)
12          -3.195   8.153  19.501                     (---*----)
                                  +---------+---------+---------+--------
                                -50       -25         0        25
```

Treatment = 8 subtracted from:

```
Treatment    Lower   Center   Upper    +---------+---------+---------+-------
 9         -21.840  -10.492   0.855               (----*---)
10         -15.318   -3.971   7.377                  (---*----)
11          -7.930    3.418  14.765                     (---*----)
12         -11.540   -0.192  11.155                   (----*---)
                                  +---------+---------+---------+-------
                                -50       -25         0        25
```

Treatment = 9 subtracted from:

```
Treatment   Lower  Center   Upper    +---------+---------+---------+---------
10         -4.826   6.522  17.869                    (----*---)
11          2.563  13.910  25.258                       (----*---)
12         -1.047  10.300  21.648                     (---*----)
                                  +---------+---------+---------+---------
                                -50       -25         0        25
```

Treatment = 10 subtracted from:

```
Treatment   Lower  Center   Upper    +---------+---------+---------+---------
11         -3.959   7.388  18.736                    (----*---)
12         -7.569   3.778  15.126                  (----*---)
                                  +---------+---------+---------+---------
                                -50       -25         0        25
```

Treatment = 11 subtracted from:

```
Treatment    Lower  Center  Upper    +---------+---------+---------+---------
12         -14.958  -3.610  7.738                (----*---)
                                  +---------+---------+---------+---------
                                -50       -25         0        25
```

Table A.2 Effect of ultrasonication time on particle size values

X1 MD:WPC & core:coating (MD:WPC(3:1, 2:2, 1:3) ; core:coating(1:8, 1:4))

X2 US time (min) (2, 5, 10)

Class Level Information

Class	Levels	Values
X1	12	1 2 3 4 5 6 7 8 9 10 11 12
X2	3	2 5 10

Number of observations read 24

Number of observations used 24

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F value	Pr>F
Model	11	9.44284800	0.85844073	52.48	<0.0001
Error	12	0.19630000	0.01635833		
Corrected Total	23	9.63914800			

R-Square	Coeff Var	Root MSE	Y Mean
0.979635	8.313273	0.127900	1.538500

Source	DF	Type I SS	Mean Square	F Value	Pr> F
X1	11	9.44284800	0.85844073	52.48	<.0001
X2	0	0.00000000			

Source	DF	Type I SS	Mean Square	F Value	Pr> F
X1	11	9.44284800	0.85844073	52.48	<.0001
X2	0	0.00000000			

Source	DF	Type III SS	Mean Square	F Value	Pr> F
X1	9	6.61372475	0.73485831	44.92	<.0001
X2	0	0.00000000			

Duncan's Multiple Range Test for Y

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

Alpha 0.05

Error Degrees of Freedom 12

Error Mean Square 0.016358

Number of Means 2 3 4 5 6 7 8 9 10 11 12

Critical Range 0 .2787 0.2917 0.2996 0.3048 0.3084 0.3110 0.3128 0.3142 0.3151 0.3157 0.3161

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	2.4375	2	1
B	2.1540	2	2
CB	2.0270	2	5
CB	2.0150	2	9
CD	1.8270	2	6
D	1.6820	2	4

D	1.6810	2	10
D	1.6020	2	3
E	1.2115	2	7
E	1.0065	2	8
F	0.4185	2	11
F	0.4000	2	12

Duncan's Multiple Range Test for Y

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

Alpha	0.05	
Error Degrees of Freedom	12	
Error Mean Square	0.016358	
Number of Means	2	3
Critical Range	0.1393	0.1458

Means with the same letter are not significantly different.

Duncan grouping	**Mean**	**N**	**X2**
A	1.96888	8	2
B	1.51800	8	5
C	1.12863	8	10

APPENDIX B

PARTICLE SIZE DISTRIBUTION DATA

MD:3 WPC:1 core:coating-1:8 US 2min

Volume(%)	D[3,2]	(ųm)
0.02		0
0.022		0
0.025		0
0.028		0
0.032		0
0.036		0
0.04		0
0.045		0
0.05		0
0.056		0
0.063		0
0.071		0
0.08		0
0.089		0
0.1		0
0.112		0
0.126		0
0.142		0
0.159		0
0.178		0
0.2		0
0.224		0
0.252		0
0.283		0
0.317		0
0.356		0
0.399		0
0.448		0
0.502		0.01
0.564		0.07

0.632	0.24
0.71	0.5
0.796	0.81
0.893	1.2
1.002	1.67
1.125	2.2
1.262	2.76
1.416	3.36
1.589	3.99
1.783	4.61
2	5.22
2.244	5.8
2.518	6.32
2.825	6.77
3.17	7.08
3.557	7.24
3.991	7.18
4.477	6.87
5.024	6.31
5.637	5.54
6.325	4.6
7.096	3.6
7.962	2.62
8.934	1.75
10.024	1.05
11.247	0.52
12.619	0.1
14.159	0.01
15.887	0
17.825	0
20	0
22.44	0
25.179	0
28.251	0
31.698	0
35.566	0
39.905	0
44.774	0
50.238	0

56.368	0
63.246	0
70.963	0
79.621	0
89.337	0
100.237	0
112.468	0
126.191	0
141.589	0
158.866	0
178.25	0
200	0
224.404	0
251.785	0
282.508	0
316.979	0
355.656	0
399.052	0
447.744	0
502.377	0
563.677	0
632.456	0
709.627	0
796.214	0
893.367	0
1002.374	0
1124.683	0
1261.915	0
1415.892	0
1588.656	0
1782.502	0
2000	0

MD:2 WPC:2 core:coating-1:8 US 2min

Volume(%) D[3,2] (ɥm)	
0.02	0
0.022	0
0.025	0
0.028	0
0.032	0
0.036	0
0.04	0
0.045	0
0.05	0
0.056	0
0.063	0
0.071	0
0.08	0
0.089	0
0.1	0
0.112	0
0.126	0
0.142	0
0.159	0
0.178	0
0.2	0
0.224	0
0.252	0
0.283	0
0.317	0
0.356	0
0.399	0
0.448	0
0.502	0.02
0.564	0.2
0.632	0.42
0.71	0.7
0.796	1.08
0.893	1.52
1.002	2.04
1.125	2.66

1.262	3.35
1.416	4.14
1.589	4.96
1.783	5.78
2	6.54
2.244	7.16
2.518	7.58
2.825	7.74
3.17	7.63
3.557	7.25
3.991	6.62
4.477	5.8
5.024	4.86
5.637	3.87
6.325	2.93
7.096	2.08
7.962	1.39
8.934	0.85
10.024	0.48
11.247	0.24
12.619	0.08
14.159	0.03
15.887	0
17.825	0
20	0
22.44	0
25.179	0
28.251	0
31.698	0
35.566	0
39.905	0
44.774	0
50.238	0
56.368	0
63.246	0
70.963	0
79.621	0
89.337	0
100.237	0

112.468	0
126.191	0
141.589	0
158.866	0
178.25	0
200	0
224.404	0
251.785	0
282.508	0
316.979	0
355.656	0
399.052	0
447.744	0
502.377	0
563.677	0
632.456	0
709.627	0
796.214	0
893.367	0
1002.374	0
1124.683	0
1261.915	0
1415.892	0
1588.656	0
1782.502	0
2000	0

MD:1 WPC:3 core:coating-1:4 US 2min

Volume(%)	D[3,2](ɥm)
0.02	0
0.022	0
0.025	0
0.028	0
0.032	0
0.036	0
0.04	0
0.045	0

0.05	0
0.056	0
0.063	0
0.071	0
0.08	0
0.089	0
0.1	0
0.112	0
0.126	0
0.142	0
0.159	0
0.178	0
0.2	0
0.224	0
0.252	0
0.283	0
0.317	0
0.356	0
0.399	0
0.448	0.23
0.502	0.89
0.564	1.29
0.632	1.82
0.71	2.34
0.796	2.87
0.893	3.41
1.002	3.94
1.125	4.44
1.262	4.92
1.416	5.33
1.589	5.64
1.783	5.83
2	5.85
2.244	5.72
2.518	5.42
2.825	5
3.17	4.49
3.557	3.94
3.991	3.38
4.477	2.85
5.024	2.39
5.637	2

6.325	1.67
7.096	1.43
7.962	1.23
8.934	1.1
10.024	1
11.247	0.91
12.619	0.86
14.159	0.79
15.887	0.76
17.825	0.72
20	0.71
22.44	0.7
25.179	0.7
28.251	0.69
31.698	0.67
35.566	0.62
39.905	0.54
44.774	0.43
50.238	0.31
56.368	0.12
63.246	0.05
70.963	0
79.621	0
89.337	0
100.237	0
112.468	0
126.191	0
141.589	0
158.866	0
178.25	0
200	0
224.404	0
251.785	0
282.508	0
316.979	0
355.656	0
399.052	0
447.744	0
502.377	0
563.677	0
632.456	0
709.627	0

796.214	0
893.367	0
1002.374	0
1124.683	0
1261.915	0
1415.892	0
1588.656	0
1782.502	0
2000	0

MD:1 WPC:3 core:coating- 1:8 US 2 min

Volume(%)	D[3,2](ɥm)
0.02	0
0.022	0
0.025	0
0.028	0
0.032	0
0.036	0
0.04	0
0.045	0
0.05	0
0.056	0
0.063	0
0.071	0
0.08	0
0.089	0
0.1	0
0.112	0
0.126	0
0.142	0
0.159	0
0.178	0
0.2	0
0.224	0

0.252	0
0.283	0
0.317	0
0.356	0
0.399	0
0.448	0.09
0.502	0.39
0.564	0.74
0.632	1.23
0.71	1.8
0.796	2.48
0.893	3.24
1.002	4.08
1.125	4.97
1.262	5.85
1.416	6.68
1.589	7.37
1.783	7.85
2	8
2.244	7.83
2.518	7.34
2.825	6.59
3.17	5.68
3.557	4.72
3.991	3.75
4.477	2.89
5.024	2.14
5.637	1.54
6.325	1.08
7.096	0.74
7.962	0.49
8.934	0.32
10.024	0.12
11.247	0
12.619	0
14.159	0
15.887	0
17.825	0
20	0

22.44	0
25.179	0
28.251	0
31.698	0
35.566	0
39.905	0
44.774	0
50.238	0
56.368	0
63.246	0
70.963	0
79.621	0
89.337	0
100.237	0
112.468	0
126.191	0
141.589	0
158.866	0
178.25	0
200	0
224.404	0
251.785	0
282.508	0
316.979	0
355.656	0
399.052	0
447.744	0
502.377	0
563.677	0
632.456	0
709.627	0
796.214	0
893.367	0
1002.374	0
1124.683	0
1261.915	0
1415.892	0
1588.656	0
1782.502	0

2000 0

MD:3 WPC:1 Core:Coating – 1:8 US 5 min

Volume(%)	D[3,2](ɥm)
0.02	0
0.022	0
0.025	0
0.028	0
0.032	0
0.036	0
0.04	0
0.045	0
0.05	0
0.056	0
0.063	0
0.071	0
0.08	0
0.089	0
0.1	0
0.112	0
0.126	0
0.142	0
0.159	0
0.178	0
0.2	0
0.224	0
0.252	0
0.283	0
0.317	0
0.356	0
0.399	0
0.448	0
0.502	0.02
0.564	0.22

0.632	0.47
0.71	0.8
0.796	1.23
0.893	1.74
1.002	2.32
1.125	3.02
1.262	3.77
1.416	4.61
1.589	5.47
1.783	6.34
2	7.11
2.244	7.74
2.518	8.12
2.825	8.19
3.17	7.93
3.557	7.32
3.991	6.42
4.477	5.35
5.024	4.2
5.637	3.08
6.325	2.1
7.096	1.29
7.962	0.73
8.934	0.29
10.024	0.12
11.247	0
12.619	0
14.159	0
15.887	0
17.825	0
20	0
22.44	0
25.179	0
28.251	0
31.698	0
35.566	0
39.905	0
44.774	0

50.238	0
56.368	0
63.246	0
70.963	0
79.621	0
89.337	0
100.237	0
112.468	0
126.191	0
141.589	0
158.866	0
178.25	0
200	0
224.404	0
251.785	0
282.508	0
316.979	0
355.656	0
399.052	0
447.744	0
502.377	0
563.677	0
632.456	0
709.627	0
796.214	0
893.367	0
1002.374	0
1124.683	0
1261.915	0
1415.892	0
1588.656	0
1782.502	0
2000	0
	0

MD:2 WPC:2 core to coating- 1:8 US 5 min

Volume(%) D[3,2]	(ɥm)
0.02	0
0.022	0
0.025	0
0.028	0
0.032	0
0.036	0
0.04	0
0.045	0
0.05	0
0.056	0
0.063	0
0.071	0
0.08	0
0.089	0
0.1	0
0.112	0
0.126	0
0.142	0
0.159	0
0.178	0
0.2	0
0.224	0
0.252	0
0.283	0
0.317	0
0.356	0
0.399	0
0.448	0.06
0.502	0.3
0.564	0.61
0.632	0.99
0.71	1.42
0.796	1.92
0.893	2.48
1.002	3.08
1.125	3.75

1.262	4.44
1.416	5.14
1.589	5.8
1.783	6.4
2	6.86
2.244	7.15
2.518	7.22
2.825	7.08
3.17	6.71
3.557	6.16
3.991	5.43
4.477	4.63
5.024	3.77
5.637	2.94
6.325	2.17
7.096	1.51
7.962	0.97
8.934	0.58
10.024	0.29
11.247	0.12
12.619	0.02
14.159	0
15.887	0
17.825	0
20	0
22.44	0
25.179	0
28.251	0
31.698	0
35.566	0
39.905	0
44.774	0
50.238	0
56.368	0
63.246	0
70.963	0
79.621	0
89.337	0
100.237	0

112.468	0
126.191	0
141.589	0
158.866	0
178.25	0
200	0
224.404	0
251.785	0
282.508	0
316.979	0
355.656	0
399.052	0
447.744	0
502.377	0
563.677	0
632.456	0
709.627	0
796.214	0
893.367	0
1002.374	0
1124.683	0
1261.915	0
1415.892	0
1588.656	0
1782.502	0
2000	0

MD:1 WPC:3 core to coating-1:4 5 min

Volume(%) D[3,2]	(ɥm)
0.02	0
0.022	0
0.025	0
0.028	0
0.032	0
0.036	0
0.04	0

0.045	0
0.05	0
0.056	0
0.063	0
0.071	0
0.08	0
0.089	0
0.1	0
0.112	0
0.126	0
0.142	0
0.159	0
0.178	0
0.2	0
0.224	0
0.252	0
0.283	0
0.317	0.03
0.356	0.5
0.399	1.12
0.448	1.75
0.502	2.41
0.564	3.06
0.632	3.7
0.71	4.29
0.796	4.83
0.893	5.29
1.002	5.66
1.125	5.9
1.262	6
1.416	5.95
1.589	5.73
1.783	5.36
2	4.83
2.244	4.22
2.518	3.53
2.825	2.84
3.17	2.2
3.557	1.64

3.991	1.16
4.477	0.8
5.024	0.54
5.637	0.36
6.325	0.27
7.096	0.23
7.962	0.23
8.934	0.25
10.024	0.28
11.247	0.32
12.619	0.34
14.159	0.36
15.887	0.39
17.825	0.43
20	0.5
22.44	0.59
25.179	0.72
28.251	0.85
31.698	0.99
35.566	1.11
39.905	1.19
44.774	1.22
50.238	1.18
56.368	1.09
63.246	0.95
70.963	0.78
79.621	0.59
89.337	0.42
100.237	0.28
112.468	0.21
126.191	0.16
141.589	0.14
158.866	0.11
178.25	0.08
200	0.04
224.404	0
251.785	0
282.508	0
316.979	0

355.656	0
399.052	0
447.744	0
502.377	0
563.677	0
632.456	0
709.627	0
796.214	0
893.367	0
1002.374	0
1124.683	0
1261.915	0
1415.892	0
1588.656	0
1782.502	0
2000	0

MD:1 WPC:3 core to coating- 1:8 5min

Volume (%)	D[3,2] (ɥm)
0.02	0
0.022	0
0.025	0
0.028	0
0.032	0
0.036	0
0.04	0
0.045	0
0.05	0
0.056	0
0.063	0
0.071	0
0.08	0
0.089	0
0.1	0
0.112	0
0.126	0
0.142	0

0.159	0
0.178	0
0.2	0
0.224	0
0.252	0
0.283	0
0.317	0.03
0.356	0.45
0.399	1.04
0.448	1.63
0.502	2.26
0.564	2.86
0.632	3.46
0.71	4.02
0.796	4.53
0.893	4.96
1.002	5.33
1.125	5.59
1.262	5.72
1.416	5.73
1.589	5.59
1.783	5.31
2	4.89
2.244	4.35
2.518	3.74
2.825	3.11
3.17	2.49
3.557	1.94
3.991	1.44
4.477	1.06
5.024	0.75
5.637	0.53
6.325	0.41
7.096	0.33
7.962	0.32
8.934	0.33
10.024	0.36
11.247	0.4
12.619	0.43

14.159	0.44
15.887	0.46
17.825	0.46
20	0.47
22.44	0.49
25.179	0.5
28.251	0.53
31.698	0.56
35.566	0.58
39.905	0.6
44.774	0.62
50.238	0.61
56.368	0.61
63.246	0.59
70.963	0.59
79.621	0.57
89.337	0.58
100.237	0.61
112.468	0.65
126.191	0.71
141.589	0.74
158.866	0.77
178.25	0.71
200	0.61
224.404	0.44
251.785	0.11
282.508	0
316.979	0
355.656	0
399.052	0
447.744	0
502.377	0
563.677	0
632.456	0
709.627	0
796.214	0
893.367	0
1002.374	0
1124.683	0

1261.915	0
1415.892	0
1588.656	0
1782.502	0
2000	0

MD:3 WPC:1 core to coating 10 min

Volume(%) D[3,2]	(ɥm)
0.02	0
0.022	0
0.025	0
0.028	0
0.032	0
0.036	0
0.04	0
0.045	0
0.05	0
0.056	0
0.063	0
0.071	0
0.08	0
0.089	0
0.1	0
0.112	0
0.126	0
0.142	0
0.159	0
0.178	0
0.2	0
0.224	0
0.252	0
0.283	0
0.317	0
0.356	0
0.399	0

0.448	0
0.502	0
0.564	0.02
0.632	0.18
0.71	0.44
0.796	0.81
0.893	1.34
1.002	2.03
1.125	2.89
1.262	3.92
1.416	5.11
1.589	6.36
1.783	7.58
2	8.59
2.244	9.27
2.518	9.48
2.825	9.19
3.17	8.4
3.557	7.24
3.991	5.85
4.477	4.41
5.024	3.07
5.637	1.95
6.325	1.11
7.096	0.54
7.962	0.17
8.934	0.05
10.024	0
11.247	0
12.619	0
14.159	0
15.887	0
17.825	0
20	0
22.44	0
25.179	0
28.251	0
31.698	0
35.566	0

39.905	0
44.774	0
50.238	0
56.368	0
63.246	0
70.963	0
79.621	0
89.337	0
100.237	0
112.468	0
126.191	0
141.589	0
158.866	0
178.25	0
200	0
224.404	0
251.785	0
282.508	0
316.979	0
355.656	0
399.052	0
447.744	0
502.377	0
563.677	0
632.456	0
709.627	0
796.214	0
893.367	0
1002.374	0
1124.683	0
1261.915	0
1415.892	0
1588.656	0
1782.502	0
2000	0

MD:2 WPC:2 core to coating-1:8 10 min

Volume(%) D[3,2]	(ɥm)
0.02	0
0.022	0
0.025	0
0.028	0
0.032	0
0.036	0
0.04	0
0.045	0
0.05	0
0.056	0
0.063	0
0.071	0
0.08	0
0.089	0
0.1	0
0.112	0
0.126	0
0.142	0
0.159	0
0.178	0
0.2	0
0.224	0
0.252	0
0.283	0
0.317	0
0.356	0.02
0.399	0.19
0.448	0.45
0.502	0.72
0.564	1.01
0.632	1.34
0.71	1.68
0.796	2.04
0.893	2.46
1.002	2.95
1.125	3.52

1.262	4.23
1.416	5.05
1.589	5.95
1.783	6.84
2	7.61
2.244	8.11
2.518	8.24
2.825	7.97
3.17	7.29
3.557	6.34
3.991	5.17
4.477	3.98
5.024	2.84
5.637	1.88
6.325	1.13
7.096	0.6
7.962	0.28
8.934	0.09
10.024	0.02
11.247	0
12.619	0
14.159	0
15.887	0
17.825	0
20	0
22.44	0
25.179	0
28.251	0
31.698	0
35.566	0
39.905	0
44.774	0
50.238	0
56.368	0
63.246	0
70.963	0
79.621	0
89.337	0
100.237	0

112.468	0
126.191	0
141.589	0
158.866	0
178.25	0
200	0
224.404	0
251.785	0
282.508	0
316.979	0
355.656	0
399.052	0
447.744	0
502.377	0
563.677	0
632.456	0
709.627	0
796.214	0
893.367	0
1002.374	0
1124.683	0
1261.915	0
1415.892	0
1588.656	0
1782.502	0
2000	0

MD:1 WPC:3 core to coating- 1:4 10 min

Volume(%) D[3,2]	(ɥm)
0.02	0
0.022	0
0.025	0
0.028	0
0.032	0
0.036	0
0.04	0

0.045	0
0.05	0
0.056	0
0.063	0
0.071	0
0.08	0
0.089	0
0.1	0
0.112	0
0.126	0.09
0.142	1.79
0.159	4.07
0.178	6.81
0.2	7.8
0.224	8.32
0.252	8.01
0.283	7.18
0.317	6.18
0.356	5.23
0.399	4.41
0.448	3.72
0.502	3.06
0.564	2.48
0.632	1.97
0.71	1.56
0.796	1.25
0.893	1.11
1.002	1.1
1.125	1.23
1.262	1.38
1.416	1.5
1.589	1.53
1.783	1.48
2	1.35
2.244	1.2
2.518	1.01
2.825	0.82
3.17	0.65
3.557	0.53

3.991	0.42
4.477	0.37
5.024	0.35
5.637	0.35
6.325	0.35
7.096	0.36
7.962	0.36
8.934	0.35
10.024	0.33
11.247	0.31
12.619	0.29
14.159	0.27
15.887	0.27
17.825	0.28
20	0.31
22.44	0.36
25.179	0.45
28.251	0.54
31.698	0.64
35.566	0.73
39.905	0.8
44.774	0.8
50.238	0.75
56.368	0.64
63.246	0.35
70.963	0.14
79.621	0.01
89.337	0
100.237	0
112.468	0
126.191	0
141.589	0
158.866	0
178.25	0
200	0
224.404	0
251.785	0
282.508	0
316.979	0

355.656	0
399.052	0
447.744	0
502.377	0
563.677	0
632.456	0
709.627	0
796.214	0
893.367	0
1002.374	0
1124.683	0
1261.915	0
1415.892	0
1588.656	0
1782.502	0
2000	0

MD:1 WPC:3 core to coating- 1:8 US 10 min

Volume(%) D[3,2]	(ɥm)
0.02	0
0.022	0
0.025	0
0.028	0
0.032	0
0.036	0
0.04	0
0.045	0
0.05	0
0.056	0
0.063	0
0.071	0
0.08	0
0.089	0
0.1	0
0.112	0

0.126	0
0.142	0
0.159	0.79
0.178	2.4
0.2	3.54
0.224	4.33
0.252	4.72
0.283	4.82
0.317	4.74
0.356	4.6
0.399	4.42
0.448	4.2
0.502	3.92
0.564	3.6
0.632	3.29
0.71	3.01
0.796	2.78
0.893	2.61
1.002	2.5
1.125	2.41
1.262	2.32
1.416	2.2
1.589	1.99
1.783	1.75
2	1.48
2.244	1.21
2.518	0.98
2.825	0.79
3.17	0.65
3.557	0.57
3.991	0.53
4.477	0.53
5.024	0.56
5.637	0.6
6.325	0.63
7.096	0.66
7.962	0.68
8.934	0.67
10.024	0.66

11.247	0.64
12.619	0.62
14.159	0.61
15.887	0.61
17.825	0.62
20	0.66
22.44	0.72
25.179	0.79
28.251	0.88
31.698	0.96
35.566	1.05
39.905	1.11
44.774	1.15
50.238	1.19
56.368	1.18
63.246	1.16
70.963	1.09
79.621	0.99
89.337	0.82
100.237	0.66
112.468	0.35
126.191	0
141.589	0
158.866	0
178.25	0
200	0
224.404	0
251.785	0
282.508	0
316.979	0
355.656	0
399.052	0
447.744	0
502.377	0
563.677	0
632.456	0
709.627	0
796.214	0
893.367	0

1002.374	0
1124.683	0
1261.915	0
1415.892	0
1588.656	0
1782.502	0
2000	0