ENCAPSULATION OF ROSEMARY ESSENTIAL OIL

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ENCAPSULATION OF ROSEMARY ESSENTIAL OIL

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ABSTRACT

ENCAPSULATION OF ROSEMARY ESSENTIAL OIL

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Encapsulation protects sensitive food ingredients against oxygen, heat, moisture and pH until they are released to the system. In addition, it can mask the unwanted taste of nutrients that are added to the foods for fortification purposes. The studies about encapsulation of essential oils in micro or nano-size are very much limited.

The objective of the study was to encapsulate the rosemary essential oil in micron size and to find the optimum coating material formulation by investigating the physicochemical properties and storage stability of microcapsules. In the wall material preparation two different ratios of maltodextrin (MD) and whey protein concentrate (WP) were used (3:1 and 1:3). The emulsions were prepared with four different core to coating ratios (1:80, 1:40, 1:20 and 1:10) and two different dextrose equivalent (DE) maltodextrins (DE:13-17 and DE:4-7). Freeze dried capsules were analyzed for their drying efficiencies, encapsulation efficiencies, surface morphologies and particle size distributions. In addition, concentrations of 1,8-cineole were determined during storage.

Increasing WP:MD ratio was found to increase both drying and encapsulation efficiencies. Also, capsules having core to coating ratio of 1:20 gave the highest drying and encapsulation efficiency. Maltodextrin with DE:13-17 was proven to have better encapsulating properties than maltodextrin with DE:4-7. Changing DE value of MD did not have any significant effect on particle size distributions and surface morphologies of the capsules. Although maltodextrin with DE value of 4-7 provided better storage stability to the capsules for the first 30 days of storage, percent retention of 1,8-cineole in these capsules were similar to the capsules containing maltodextrin with DE value of 13-17 at the end of 40 days of storage.

Keywords: Microencapsulation, rosemary essential oil, whey protein concentrate, maltodextrin, dextrose equivalence

ÖZ

BİBERİYE YAĞININ ENKAPSÜLASYONU

Turasan, Hazal
Yüksek Lisans, Gıda Mühendisliği Bölümü
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Enkapsülasyon oksijen, ısı, nem ve pH'a karşı hassas gıda bileşenlerini sisteme salınıncaya kadar korur. Ayrıca, gıdaya zenginleştirmek amaçlı katılan besinlerin istenmeyen tadını maskeler. Uçucu yağların mikro ve nano-boyutta enkapsülasyonu ile ilgili olan çalışmalar oldukça sınırlıdır.

Bu çalışmanın amacı, biberiye uçucu yağının mikron boyutta enkapsülasyonu ve kapsüllerin fizikokimyasal özellikleri ile depolama sırasındaki dayanıklılığının incelenerek optimum kaplama maddesi formülasyonunun bulunmasıdır. Kaplama maddesi hazırlanmasında iki farklı maltodekstrin ve peynir altı suyu protein konsantresi oranı kullanılmıştır (3:1 ve 1:3). Emülsiyonlar dört farklı uçucu yağ/kaplama maddesi oranında (1:80, 1:40, 1:20 ve 1:10) ve iki farklı dekstroz eşdeğerli (DE) maltodekstrin (DE:13-17 ve DE:4-7) ile hazırlanmıştır. Dondurmalı kurutucuda kurutulan kapsüllerin kurutma verimleri, enkapsülasyon verimleri, yüzey morfolojileri ve parçacık boyut dağılımları analiz edilmiştir. Ayrıca depolama sırasında, 1,8-sineol konsantrasyonu tespit edilmiştir.

Artan peynir altı suyu protein konsantresi/maltodekstrin oranının hem kurutma hem de enkapsülasyon verimliliğini arttırdığı görülmüştür. Ayrıca, 1:20 uçucu yağ/kaplama maddesi oranına sahip olan kapsüller en yüksek kurutma ve enkapsülasyon sonuçlarını vermiştir. Kısaca, 13-17 dekstroz eşdeğerli maltodekstrinin 4-7 dekstroz eşdeğerli maltodekstrinden daha iyi enkapsülasyon özelliğine sahip olduğu kanıtlanmıştır. Maltodekstrinin DE değerinin değiştirilmesinin kapsüllerin parçacık boyutu dağılımları ve yüzey morfolojileri üzerinde önemli bir etkisinin olmadığı görülmüştür. DE değeri 4-7 olan maltodekstrin depolamanın ilk 30 gününde kapsüllere daha iyi dayanıklılık özelliliği sağlamasına rağmen, depolamanın sonunda (40 gün) bu kapsüllerdeki 1,8-sineolun tutulma yüzdesi 13-17 DE değerli maltodekstrin içerin kapsüllerinkiyle yakın hale gelmiştir.

Anahtar Kelimeler: Enkapsülasyon, biberiye uçucu yağı, peyniraltı suyu protein konsantresi, maltodekstrin, dekstroz eşdeğerliği

To my beloved mother…

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INTRODUCTION

1.1 Functional foods

During the last decades, consumer's demands from the food production industry have remarkably increased. People no longer see the food to appease the hunger but as a source to get the required nutrients which are supposed to help with the nutrition-related diseases and contribute to both physical and mental well-being of individuals (Bigliardi & Galati, 2013). This trend forced the food researchers and the producers to concentrate more on the production of foods that meets the requirements of humans for healthier lives (Bigliardi & Galati, 2013).

The term "functional food" first appeared in 1970's in Japan; it was only a decade later that this term was introduced to Europe. Although the term "functional foods" has been used only recently, the roots could be traced back to a thousand years earlier, when foods were being used to treat people in China (Guo, 2008). There are several definitions for functional foods. According to Riemersma, (1996), the definition of functional foods is as simple as "the food products marketed with the message of the benefit to health". A more complex definition of functional foods says that functional foods are the foods which are derived from the naturally occurring substances which can and should be consumed as part of the daily diet and which regulates a specific body function (Smith, Marcotte, & Harman, 1996). Bigliardi & Galati (2013) defines functional foods with their objectives, such as, functional foods are the foods that improve the general conditions of the body and decrease the risk of some diseases and which can also be used to cure some illnesses. Ozen, Pons, & Tur, (2012) and Roberfroid (2002)

agree that functional foods can not be defined with a single definition since functional foods should be acknowledged as a concept including a variety of foods either classified as nutrients or not and as either affecting a variety of body functions or just reducing the risk of illnesses (Roberfroid, 2002).

Recently, functional foods are being produced in many different areas. Today, the class of functional foods in the market mainly consist of dairy products, including low-fat milk, skim milk, probiotic milk and yoghurt; coffee and tea; cholesterol lowering products, such as cholesterol reducing margarine; fermented drinks, such as red wine; fiber-enriched cereals; bakery products, such as omega-3 enriched or whole grain breads; soy products; baby food; vitamin-enriched fruit juices; seafood such as fish oil; poultry products; herbs and spices and many other (Bigliardi & Galati, 2013; Onwulata, 2013; Ozen, Pons, & Tur, 2012). Sloan (2000) and Spence (2006) classified functional foods under four categories; a) foods fortified with the addition of other nutrients, such as fruit juices enriched with calcium and folic acid; b) foods enriched with extra substances which normally are not seen in a particular food, such as probiotics and prebiotics; c) foods from which some harmful substances are removed and replaced by more healthier ones, such as ice-cream with additional fiber as fat-releasers and d) foods with increased amount of specific constituents, such as eggs with enhanced omega-3.

The benefits of functional foods on human health are innumerous. Makinen-Aakula (2006) classified the health benefits of functional foods in three main groups; direct health benefits, decreased disease risks and improved life conditions. Here are some examples of benefits of functional foods. Jones & Jew (2007) found that the addition of lactic acid bacteria and bifidobacteria to foods as probiotics increases the gastrointestinal health and the immune functions. The omega-3 enriched egg seems to have reduced the possible formation of blood clots and controlled the blood pressure (Sirõ et al., 2008). Tammsaar (2007) showed that the cholesterol lowering beverages not only reduces the cholesterol levels but also stimulates the antioxidant functions and inhibits the deformation of bones.

With the advanced technology in food production industry, there are now different techniques to produce functional foods. Betoret et al., (2011) identified and categorized the technologies for the development of functional foods in three groups; traditional methods which are being used in regular food processing, technologies which are designed to prevent the impairment of some active compounds and technologies to design personalized functional foods. Especially to overcome the problem of active compound deterioration novel technologies are adopted rather than the traditional ones. This relatively more advanced techniques are based on the idea of coating the desired active compound, such as probiotics, before implanting to the food to prevent the loss of necessary substances (Bigliardi & Galati, 2013). Some of this entrapment techniques are microencapsulation, coating with edible films or vacuum impregnation. The details of microencapsulation technique will be examined in subsequent sections.

1.2 Essential Oils

There are different definitions for essential oils. Valgimigli (2012) defined essential oils as concentrated hydrophobic liquids having volatile components, which gives the aroma to the plant it is extracted from. It could also be stated that essential oils are a collection of ethereal lipophilic compounds in a liquid form, obtained from aromatic plants by different hydro or steam distillation techniques (Shah, Davidson, & Zhong, 2012; Amorati, Foti, & Valgimigli, 2013; Garcia, Tonon, & Hubinger, 2012). Although essential oils are simply referred as "the oil of the plant they are extracted from", by some people this expression could be insufficient since the term could be confused with the other non-volatile and non-fragrant edible vegetable oils such as soy oil, corn oil or other seed oils (Valgimigli, 2012). Prof. Dr. Gerhard Buchbauer suggested an alternative definition for essential oils: "Essential oils are more or less volatile substances with more or less odorous impact, produce either by steam distillation or dry distillation or by means of a mechanical treatment from one single specie" (Başer

& Buchbauer, 2010). Briefly, essential oils are the liquid secondary metabolites which are synthesized by various organs of aromatic herbs such as buds, flowers, leaves, stems, branches or seeds and characterized by strong odors and usually clear (uncolored) appearances (Andrade, Barbosa, & Probst, 2014).

The history of the discovery of essential oils remains uncertain. However, the history of the development of essential oils could be tracked back to five or six thousand years (Valgimigli, 2012). In the pre-Christian times, the essential oils were mainly used for their aromatic and medical properties. It is known that before 3000 BC, the Egyptians used the essential oil to perfume their sanctuaries or in ritual ceremonies like embalming the dead bodies (Valgimigli, 2012). Due to the lack of other cleaning alternatives like soaps, essential oil were also used as cleansing agents for hair and body. Greeks were one of the pioneers of the usage of essential oils in food industry; they used essential oils in the production of aromatic vinegars, wines and breath-refreshing gums. Cosmetics was another field of essential oil usage in the history. Especially, by French, the aromatic oils of lemon, rosemary, chamomile or thyme were employed as essences in the perfume preparation or used as aromatic additives in body creams (Valgimigli, 2012).

It is also believed that the first steps of the distillation process was taken in Egypt, Persia and India which is considered to be the basis of the production of the essential oils (Guenther, 1948). Especially in the 9th century BC, the production of aromatic oils were highly improved due to the advances in distillation techniques in the Arabic region (Surburg & Panten, 2006; Valgimigli, 2012). In the following years, the development of essential oil industry shows correlation with the development of human civilization (Valgimigli, 2012). The discoveries in the 17th century accelerated the evolution of essential oils industry. After the investigations of the constituents of the essential oils, such as the discoveries of hydrocarbons and terpenes, and the foundation of advanced distillation techniques, the discipline of essential oils gained pace (Başer & Buchbauer, 2010).

In today's world, the properties of essential oils are known better and due to these properties, their usage areas have been extensively enlarged. At the present, of 3000 essential oils known, 300 different type of essential oils or some components of them are being used commercially in the production of perfumes, make-up products, as sanitizers, food additives and natural remedies in aromatherapy and in the field of agriculture or medicine (Bakkali et al., 2008). According to the data collected based on the average prices of essential oils in the market in 2007, the largest world consumer of essential oils is the flavor industry (Başer & Buchbauer, 2010). For instance, the leading essential oils with the highest consumption rates are orange and cornmint oil, whose major applications are soft drinks, chewing gums and sweets oral care and fragrances (Başer & Buchbauer, 2010).

The functional properties of essential oils mainly depend on the chemical compositions of essential oils (Valgimigli, 2012). These natural aromatic mixtures may be composed of 20-60 compounds, which are at different concentrations from each other (Bakkali et al., 2008). The majority of the total content is usually composed of two or three main compounds with quite high percentages, where the remaining part is composed of other metabolites with trace amounts (Valgimigli, 2012). For example, the terpenes thymol, carvacrol, γ-terpinene could be specified as the major constituents of oregano (*Origanum compactum*) essential oil and thyme (*Thmus officinalis* L.) essential oil; menthone, iso-menthone and menthol are the major components of peppermint (*Mentha piperita* L.) essential oil; 1,8-cineole, α-pinene and camphor are the main terpenoids composing rosemary (*Rosemarinus officinalis* L.) essential oil (Rahimmalek & Goli, 2013; Baranauskiené et al., 2007; Verma, Padalia, & Chauhan, 2012; Zawirska-Wojtasiak & Wasowicz, 2009).

Depending on their chemical compositions, essential oils possess some biological activities either on the plants or human systems. Their roles in the plants are mainly protecting the plants from pathogens and predators by their antibacterial and antifungal activities. Since the terpenoids and other phenolic compounds are highly toxic for most of the insects and highly deleterious for fungal infections,

they are very effective in repelling these harmful agents (Valgimigli, 2012). Also by reducing the appetite for herbivores or enhancing the aromatic scent of the plant, essential oils contribute to repelling the unwanted animals and attracting the pollinators (Bakkali et al., 2008; Valgimigli, 2012).

The biological effects of essential oils in human body could be analyzed on a larger spectrum. Essential oils are known to possess anti-inflammatory, antinociceptive, anticancer, antiviral, antimicrobial, analgesic, sedative, antiphlogistic and antioxidant activities among human bodies (Valgimigli, 2012; Başer & Buchbauer, 2010; Arvind & Vyas, 2013; Bakkali et al., 2008; Andrade, Barbosa, & Probst, 2014; Yanishlieva, Marinova, & Pokorny, 2006; Amorati, Foti, & Valgimigli, 2013).

Chamomile oil, sage oil, rosemary oil and savory oil are some examples of essential oils that are reported to have anti-inflammatory effects (Adorjan & Buchbauer, 2010). Maham, Moslemzadeh, & Jalilzadeh-Amin, (2014), Paula-Freire et al., (2013) and Liangab, Huanga, & Wang (2012) investigated and proved the antinociceptive effect of Tarragon (*Artemisia dracunculus*) essential oil, *Ocimum gratissimum* L. (Lamiaceae) Essential Oil and *Illicium lanceolatum* essential oil respectively. The latest studies showed that *Melissa officinalis* L., *Lynderia strychnifolia* L., *Cyperus rotundus* C., Euphorbiaceae *Croton flavens* L., basil (*Ocimum basilicum* L.) are only some of the essential oils that has shown promising results in the field of treatment of tumors by their constituents like α-cyperene, β-caryophyllene and other anticarcinogenic compounds (Başer & Buchbauer, 2010). Andrade, Barbosa, & Probst (2014) has investigated the antimicrobial effect of twenty essential oils including bergamot (*Citrus aurantium bergamia*), black pepper (*Piper nigrum*), cinnamon (*Cinnamomum cassia*), eucalyptus *(Eucalyptus globulus*), orange (*Citrus aurantium dulcis*), and rosemary (*Rosmarinus officinallis*), ginger (*Zingiber officinalis*), lavender (*Lavandula officinalis*), lemongrass (*Cymbopogon schoenanthus*).

The most abundant data collected about essential oils may be about their antioxidant activity. The antioxidant effect of essential oils has been verified by

several studies (Amorati, Foti, & Valgimigli, 2013; Yanishlieva, Marinova, & Pokorny, 2006; Bakkali et al., 2008; Valgimigli, 2012). According to Amorati, Foti, & Valgimigli (2013), the essential oils of oregano (*Origanum vulgare* L.), thyme (*Thymus vulgaris* L.), clove (*Syzygium aromaticum* L.), bush-basil (*Ocimum minimum* L.) and black cumin (*Nigella sativa* L.) are showing better antioxidant effects compared to other oils like sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.), green anise (*Pimpinella anisum* L.), tarragon (*Artemisia dracunculus* L.) and many others. This better effect could be related to the higher concentrations of antioxidant terpenes found in oregano, thyme, clove, bush-basil and black cumin oils, especially thymol and carvacrol (Valgimigli, 2012).

The compositions of terpene compounds in the essential oils are believed to the chemical nature of them (Bajpai, Shukla & Sharma, 2013). The analeptic, antibacterial, antifungal and antioxidant activities are well known (Başer & Buchbauer, 2010). Terpenes could be found in the structures of mono-, sesqui-, hemi-, di-, tri-, and tetraterpenes and they are found in the forms of hydrocarbons or derivatives of oxygen molecule (Bajpai, Shukla & Sharma, 2013). The monoterpenes including compounds like carvacrol, thymol, camphor, limonene, 1,8-Cineole, α-Terpineol or pinene have been reported as antioxidants by many (Valgimigli, 2012; Başer & Buchbauer, 2010; Amorati, Foti & Valgimigli, 2013). According to the findings of Valgimigli (2012) and Amorati, Foti & Valgimigli (2013), the essential oils showing the best antioxidant properties are the oils containing thymol, carvacrol and eugenol with very high concentrations.

The derivation of essential oils is mainly done directly from the plant itself (Valgimigli, 2012). Different extraction techniques with changing solvent power or the selection of the extraction method with appropriate extraction media enables obtaining both the volatile and non-volatile compounds from the essential oils. (Valgimigli, 2012). Among the traditional extraction methods of essential oils, distillation technique, is recognized as the most common extraction method used (Guenther, 1948; Arvind & Vyas, 2013). Başer & Buchbauer (2010) stated that steam or water distillation technique is the most frequently used method in

the production of volatile oils. As Guenther (1948) mentions, the beginning of essential oils is considered to have begun with the invention of distillation technique which dates back to pre-Christian era and so far it is considered as the simplest extraction method. Besides distillation, the other traditional techniques used to produce essential oils are mainly solvent extraction methods, such as maceration or enfleurage (extraction by using fat) and cold pressing technique, which is a simple mechanical method usually used with an additional separation process (Valgimigli, 2012). With the development of technology, modern extraction method have developed. The main aim of the modern techniques is to eliminate the disadvantages of traditional methods like shortening the time or saving the energy (Valgimigli, 2012). Valgimigli (2012) collects the modern extraction methods under three main categories: headspace methods, modified distillation methods and modified solvent extractions. Among the modern extraction techniques microwave-assisted extraction is considered to be the best method with a high yield of extraction and the potential of being the pioneer for new techniques to be developed (Zhi-ling et al., 2011).

1.3 Rosemary Essential Oil

Rosemary (*Rosmarinus officinalis* L.) is a long-lasting evergreen aromatic herb which has needle-like leaves (Fig. 1.1) (Bousbia, et al., 2009; Le romarin, 2014). The plant rosemary is mainly grown in Mediterranean countries such as Italy, Spain, Turkey, Egypt, Portugal, Greece, France, and North Africa (Atti-Santos, et al., 2005). It is also cultivated in other countries like Argentina, Brazil or Uruguay (Miguel et al., 2007). *Rosmarinus officinalis* is a member of *Rosmarinus* genus (Valgimigli, 2012). This genus is under the category of *Lamiaceae* family, which contains approximately 200 genera with 3500 different species (Lo Presti, et al., 2005). This particular species is the most investigated species when compared to other species in the genus *Rosmarinus* such as *Rosmarinus eriocalyx, Rosmarinus lavandulaceus* or *Rosmarinus tomentosus* (Valgimigli, 2012). *Rosmarinus*

officinalis is a fragrant shrub with the characteristics of intense pleasant odor, lavender-like leaves with the color dark green and its flowering season could last from April to August (Lo Presti, et al., 2005). The height of the plant can reach up to 1.5 m (Atti-Santos, et al., 2005). The natural habitat of this plant is Mediterranean climate where the dominant weather is warm and dry (Serrano et al., 2002) and it is adapted to sandy, chalky soil type with less humus (Flamini et al., 2002).

Figure 1.1 *Rosmarinus officinalis* L.

Lo Presti et al., (2005) state that the highest quality of rosemary essential oil is obtained from the leaves of the plant, where the majority of oil glands are located. The rest of the essential oil is obtained from flowering aerial tops, twigs, and flowers of the plant (Flamini et al., 2002).

As mentioned previously, the aromatic herbs and their essential oil have been used for ages for many purposes. The oil of *Rosmarinus officinalis* is no different than others; the usage of the oil of rosemary dates back to 1500s (Guenther, 1948). According to Bousbia, et al. (2009), before refrigeration was invented rosemary oil was used for food preservation purposes as well as medical antiseptic, and astringent purposes. Another area of usage of the essential oil was

cuisines in the ancient times (Bousbia, et al., 2009). Through the years, the utilization area of rosemary oil has not been changed much. In folk medicine today, rosemary oil is used in the treatment of nervous system illnesses and blood circulation diseases (Lo Presti, et al., 2005). It is applied to the patients with chronic weaknesses or vascular disorders (Lo Presti, et al., 2005). Rosemary oil is also adopted as disinfectant and insecticide due to its antipathogenic activities (Lo Presti, et al., 2005). Also due to its pleasent aroma, rosemary essential oil is widely used as the fragrance component in the cosmetic industry. The primary products that this oil is used are lotions, perfumes, soaps and creams (Flamini et al., 2002). The oil of rosemary is still used for culinary purposes as well; it is used as flavoring meats and sauces (Lo Presti, et al., 2005) and used for prolonging the shelf-life of the foodstuff (Bousbia, et al., 2009).

There are many studies conducted on the chemical compositions, biological properties or the usage of rosemary essential oil (Lo Presti, et al., 2005).

The chemical composition of rosemary essential oil has been analyzed by many researches (Socaci, Tofană, & Socaciu, 2008; Bozin et al., 2007; Fernandes, et al., 2013; Usai, et al., 2011). Although the concentrations of its compounds usually vary depending on the growing habitat, the major constituents are well-known (Socaci, Tofană, & Socaciu, 2008). Rosemary oil mainly involves monoterpenes in its composition, which include 1,8-cineole, α-pinene, camphor, camphene, limonene, borneol, myrcene, and *p*-cymene (Katerinopoulos et al., 2005). All the therapeutical features of rosemary oil, its anti-inflammatory, antimicrobial, antioxidant effects come from these phenolic constituents (Başer & Buchbauer, 2010). Fig. 1.2 shows the chemical structures of major components of rosemary essential oil.

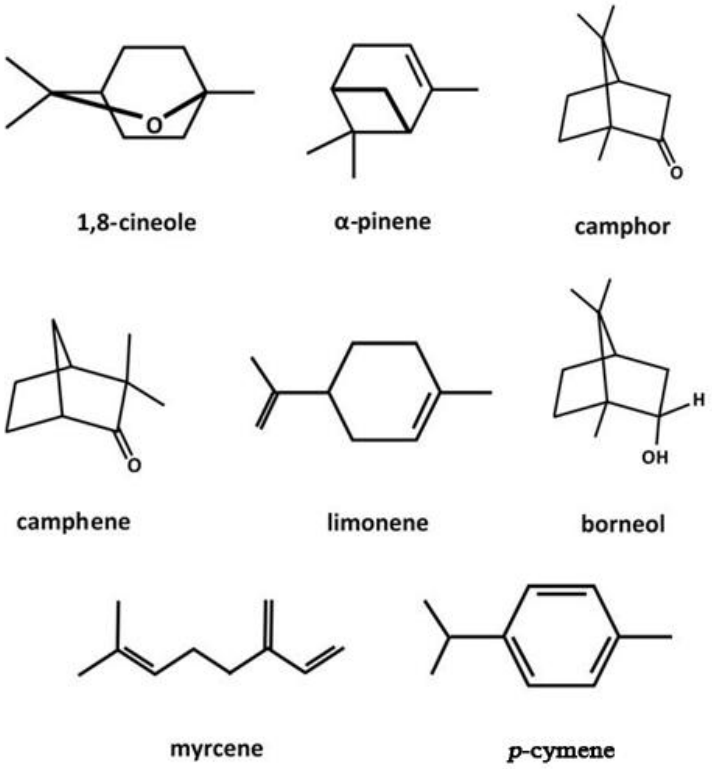

Figure 1.2 Chemical structures of major components of rosemary essential oil

In the literature, rosemary essential oil is frequently investigated for its biological activities. Among these activities, the antioxidant activity of rosemary essential oil is perhaps the characteristic that is most widely investigated (Erkan, Ayrancı, & Ayrancı, 2008; Ramireza et al., 2004; Yanishlieva, Marinova, & Pokorny, 2006; Thorsen & Hildebrandt, 2003; Kadri, et al., 2011; Amorati, Foti, & Valgimigli, 2013; Bozin et al., 2007).

As mentioned, the antioxidant effect of rosemary essential oil is based on the phenolic constituents of the oil, since almost all the phenolic compounds are proven to show antioxidant effect (Valgimigli, 2012). Among these phenolics, specifically the group of tocopherols and flavonoids including carnosic acid, carnosol, rosmanol, rosmadiphenol, rosmarinic acid and 1,8-cineol evidenced to

exhibit the antioxidant effect of rosemary essential oil (Ramireza et al., 2004; Bozin et al., 2007; Başer & Buchbauer, 2010; Yanishlieva, Marinova, & Pokorny, 2006; Erkan, Ayrancı, & Ayrancı, 2008).

Rosemary oil is also well-known with its antimicrobial features (Bozin et al., 2007; Issabeagloo et al., 2012; Valero & Salmeron, 2003). According to Valgimigli (2012), *Rosmarinus officinalis* L. was found to be more active against the Gram (+) type of bacteria when compared to Gram (-) bacteria. Romeo et al. (2008) also examined the inhibitory effect of rosemary essential oil against some food pathogens including *Staphylococcus aureus, Listeria innocua* and *Escherichia coli.* According to Valgimigli (2012), the antimicrobial action-related components of essential oil are carvacrol, carvone, cinnamaldehyde, eugenol and thymol, through which rosemary oil show its antimicrobial action.

Other than antioxidant and antimicrobial action, rosemary essential oil also exhibits pediculicidal, aromatherapeutical and anticarcinogenic activities (Başer & Buchbauer, 2010).

1.4 Microencapsulation

As Ghosh (2006) mentions, the terms "product" or "a component of a product" are insufficient to define microencapsulation, since it could only be described as a process. It could be defined as a process in which, small solid particles, liquid components or gaseous materials such as bubbles are coated by or entrapped within another inert shell material which isolates and protects the core material from environmental factors (Zhu et al., 2012; Kuang, Oliveira, & Crean, 2010; Ghosh, 2006; Desai & Park, 2005). The first applications of microencapsulation were used in the paper industry where dyes were capsulated for copying purposes (Ghosh, 2006). It was no later than that microencapsulation was used in pharmaceutical production. However, only for the last couple decades, the

applications of microencapsulation are employed in food and agricultural industries widely (Ghosh, 2006).

In the literature, there are different expressions to define both the capsulated material and the material which is used to form the capsule (Zhu et al., 2012; Ghosh, 2006; McClements, 2012). The entrapped material is called active component, core material, active agent, intrinsic part, internal phase, payload or the nucleus (Kroschwitz & Seidel, 2005; McClements, 2012; Zhu et al., 2012). The common encapsulated components in the food industry are listed in Table 1.1 (McClements, 2012).

Table 1.1 Common active agents used in food industry

Name	Example
Flavors	Citrus oil
Antimicrobials	Essential oils
Antioxidants	Carotenoids
Minerals	Iron
Vitamins	Vitamin D
Probiotics	Lactic Acid Bacteria

Different terms used for the material in which the core material is entrapped are coating material, shell material, wall material, carrier, encapsulant, membrane or the extrinsic part (Ghosh, 2006; Kroschwitz & Seidel, 2005; Zhu et al., 2012). The

matrix in which the core material is enclosed is also responsible from the release mechanism of the active component (Kuang, Oliveira, & Crean, 2010).

Microencapsulation is a very advantageous technique for food processing, especially for the encapsulation of flavors and fragrances (Zhu et al., 2012). The main advantages microencapsulation are protecting the susceptible and unstable materials from environmental factors, enhancing the processibility of them, controlling the release mechanism of the core materials by providing targetted and timed release, masking the undesired odor or taste, making it easier to handle the active compound by modifying the physical characteristics of it, providing a desired dilution or separating components from each other within a mixture (Kuang, Oliveira, & Crean, 2010; Ghosh, 2006; Desai & Park, 2005).

Depending on their structure, morphologies of microcapsules can be classified under different groups. Although it may differ from one to another, there are mainly four groups of formation; a) single core-shell in which a single type of wall surrounds one single type of core material, b) multi-walled type in which a single type of ingredient is covered with multiple coatings, c) polynuclear form in which many cores are enclosed within a shell and d) matrix encapsulation in which the core material is homogenously distributed among the shell material. Fig. 1.3 represents the morphologies of microcapsules (Zhu et al., 2012; Kuang, Oliveira, & Crean, 2010; Ghosh, 2006; Desai & Park, 2005; Kroschwitz & Seidel, 2005).

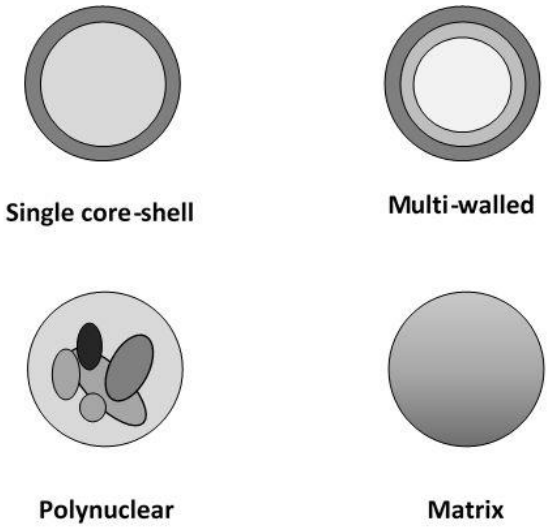

Figure 1.3 Morphologies of microcapsules

With the advance technology, there are now various techniques used for microencapsulation. From those, the most commonly employed techniques are spray drying, lyophilization, extrusion, spray-cooling, spray-chilling, fluid-bed coating, coacervation, centrifugal techniques and liposome entrapment (Desai & Park, 2005; Ghosh, 2006). The techniques used for this study will be explained later.

1.4.1 Coating Materials

The formulation of encapsulant has a great effect on the functional properties of the microcapsules and it affects the method of encapsulation (Zhu et al., 2012). Ghosh (2006) underlines the importance of the compatibility of core material and coating material since it enhances the efficiency of microencapsulation process.

According to Matsuno & Adachi (1993), especially for the encapsulation of lipids, the extent of the protection depends on the nature of the coating materials. For the coating material to give the highest encapsulation efficiencies, it has to give no reaction with the active agent used, it has to be easily treatable such as it has to exhibit low viscosity in high concentrations, it has to show good emulsion stability properties and it has to have good solubility property for the release of the core material (Zhu et al., 2012). The common coating materials used for the encapsulation of volatile food components are carbohydrates, gums, proteins, lipids and cellulose which are listed more detailed in Table 1.2 (Desai & Park, 2005).

Table 1.2 Coating materials used in the encapsulation of volatile food materials

Category	Coating Materials	Widely used methods
Carbohydrate	Starch, maltodextrins, chitosan, corn syrup solids, dextran, modified starch, cyclodextrins	Spray drying, freeze drying, extrusion, coacervation, inclusion complexation
Cellulose	Carboxymethylcellulose, methyl cellulose, ethylcellulose, celluloseacetate-phtalate, celluloseacetate-butylate-phtalate	Coacervation, spray drying and edible films
Gum	Gum acacia, agar, sodium alginate, carrageenan	Spray drying, syringe method (gel beads)
Lipids	Wax, paraffin, beeswax, diacylglycerols, oils, fats	Emulsion, liposomes, film formation
Protein	Gluten, casein, gelatin, albumin, peptides	Emulsion, spray drying

In the encapsulation of flavors, carbohydrates and mostly maltodextrins are used frequently (Runge, 2004). Maltodextrins are a subgroup of carbohydrates which are formed by the hydrolysis of starch (Chronakis, 1998). The average degree of the available sugars with the potential of hydrolysis are measured with sugars with reducing end groups and is denoted by "dextrose equivalence" (Runge, 2004) (Chronakis, 1998). Maltodextrins are hydrolyzed starches with a dextrose equivalence lower than 20 (Chronakis, 1998). Not only maltodextrins are soluble in water unlike the native starches, but also they form low viscosity solutions which makes them very easy-to-handle coating materials (Chronakis, 1998) (Runge, 2004). Maltodextrins lack surface-active properties and this leaves them unadsorbed at the interfaces of core material and shell material (Sheu & Rosenberg, 1998). This lack of emulsification characteristics makes maltodextrins be used with additional coating materials with emulsification properties to form stable emulsions (Sheu & Rosenberg, 1998). In addition, it is feasible to use maltodextrins as coating materials in the encapsulation of volatile components since they have excellent oxygen-blocking properties (Runge, 2004). In their studies Hogan et al. (2001) showed that the encapsulation efficiency of soy oil increased with the increasing dextrose equivalence of maltodextrins. This also matches to the findings of Shah et al., (2012) who found an increasing retention of thymol with increasing dextrose equivalence. According to the results of Sankarikutty et al. (1988), the surface oil content of cardamom oil microcapsules prepared with gum Arabic/maltodextrin conjugates is significantly less than the ones prepared with maltodextrin only.

Whey protein powders are important compounds used in encapsulation techniques (Whetstine, Croissant, & Drake, 2005). Whey protein concentrate and whey protein isolate are the products of process liquid whey with protein concentrations of 35-80% and >90%, respectively (Whetstine, Croissant, & Drake, 2005). The characteristics of having small molecular weight and more spherical shapes and showing low viscosities even in high concentrations make whey proteins high performance coating materials (Vardhanabhuti & Foegeding, 1999). Many investigators reported whey protein as an effective encapsulating agent

(Rosenberg & Sheu, 1996; Young, Sarda, & Rosenberg, 1993; Moreau & Rosenberg, 1996). Also the effective performance of whey protein isolate as a barrier against oxidation of microencapsulated orange essential oil is proven by (Kim, Morr, & Schenz, 1996). In addition, the high surface activity properties makes whey proteins excellent emulsifiers (Runge, 2004). Thus, it is shown by many researchers that, especially in the microencapsulation of essential oils, whey protein exhibits better efficiencies when it is combined with carbohydrates in the preparation of coating materials (Akhtar & Dickinson, 2007; Young, Sarda, & Rosenberg, 1993; Sheu & Rosenberg, 1998; Bylaitë, Venskutonis, & Maþdþierienë, 2001). Akhtar & Dickinson (2007) found that the conjugate of whey protein with maltodextrin has shown good emulsification properties and reduced droplet sizes in emulsification of either triglyceride oil or orange oil, and it has shown better solubility when compared to whey protein alone. Bylaitë, Venskutonis, & Maþdþierienë (2001) compared whey protein and skimmed-milk powder with their combination of maltodextrin and concluded that whey protein conjugate has higher retention rates of caraway essential oil. The reason of these effective results is that whey proteins act as emulsifying and film-forming agents in microencapsulation and carbohydrates act as fillers and matrix-forming agents in the process (Sheu & Rosenberg, 1998).

1.4.2 Homogenization Techniques

Emulsification is one of the most crucial steps in microencapsulation technology since it affects many results, such as encapsulation efficiency, product stability, structural properties, product rheology, flavor holding capacity or appearance. Among various homogenization methods, high-shear homogenization, ultrasonic homogenization and microfluidization are the most commonly used techniques in food industry (McClements, 2005).

1.4.2.1 Pre-homogenization

During the preparation of beverage emulsions, pre-homogenization is an important step since a premixed emulsion has smaller droplet size and shows better efficiency results (Pandolfe, 1995). One of the most common devices used in pre-homogenization step is high-speed mixers (Pandolfe, 1995). In food industry, these type of mixers are the most commonly used devices and they are mostly used in direct homogenization of oil and water phases (McClements, 2005). The reason why these high-shear homogenizers are considered to be the most effective type of homogenizers is the fact that they require low energy than other homogenization methods (Chong-hao, Dong & Li-jun, 2014). High-speed mixers are not only effective in reducing the droplet size of the emulsions but also very effective in controlling it (Salager, et al., 2004).

During the process, the components to be mixed can be added into the vessel either at the beginning of the process or they can be added sequentially during mixing. The type of the addition affects the dispersion rate and homogenization times (McClements, 2005).

Figure 5 shows the mechanism of a typical high-speed homogenizer. The mixing head rotates rapidly, usually up to 3600 rev/min, and generates a combination of longitudinal, rotational and radial velocity gradients in the agitated mixture. This rotation disrupts the interfaces between the oil and the liquid phases, causing the liquids to become intermingled and eventually breaking the larger droplets into smaller ones. At the end of a typical high-shear homogenization process, the droplet size of the emulsion is reduced to a range of 2-10 μm in diameter (McClements, 2005).

Since high-shear homogenizers are usually used prior to other homogenization techniques, there are only a limited number of research investigating only the effect of high-shear homogenizers. From those for instance, Chong-hao, Dong, & Li-jun (2014) investigated the effect of ultra-turrax homogenizer at seven different rotational speed on rheological and structural properties on SPI gels and

concluded that the Z-average values of protein particles of SPI dispersions decreased significantly with the increasing rotational speed. Fradette, Brocart, & Tanguy (2007) compared the effects of ultra-turrax homogenizer and three other mechanical agitation types on the water-in-oil emulsion diameters. They found that especially between 60-70% water content, ultra-turrax creates smaller droplets when compared to other low energy agitators (Fradette, Brocart, & Tanguy, 2007).

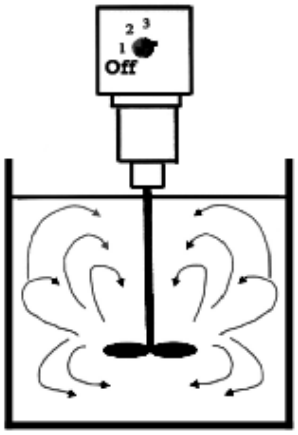

Figure 1.4 High-speed homogenizer

1.4.2.2 Ultrasonic Homogenization

The appearance of ultrasound emulsification in scientific world took place in Switzerland, where the patent of it was granted for the first time (Abismail et al., 1999). Ever since there has been a growing interest in using ultrasonication in emulsification particularly in the fields of food and pharmaceuticals (Ertugay, Şengül, & Şengül, 2004; Kentish, et al., 2008; Freitas et al., 2006). Depending on the utilization such as industrial or laboratory scale, different methods of ultrasonication is applied (McClements, 2005). Two most commonly used

methods are piezoelectric transducers, which is the bench-top type of homogenizer and liquid jet generator, which is the industrial type of ultrasonicator (McClements, 2005). In piezoelectric transducer homogenizer, the ultrasonic waves are radiated from the tip of the transducer into the surrounding liquid sample and breaks up the droplets into smaller ones by generating intense pressure and shear gradient (McClements, 2005). Fig. 1.5 shows a typical lab scale ultrasonic homogenizer.

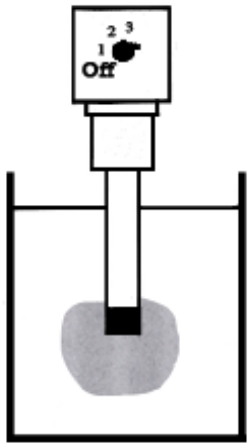

Figure 1.5 Ultrasonic homogenizer

The working principle of ultrasonic homogenization is creating cavitation (Jafari, He, & Bhandari, 2006). Jafari, He, & Bhandari (2006) defines cavitation as the formation and the collapse of vapor cavities in the flowing liquid. Due to the local velocity changes in the surrounding liquid sample, the local pressure is reduced to the vapor pressure which leads to the formation of vapor cavities (Jafari, He, & Bhandari, 2006). The collapse of these cavities helps the ultrasonic waves radiate through the solution starting from the tip of the probe (Jafari, He, & Bhandari, 2006). Briefly, the emulsification by ultrasonic homogenizer occurs in two stages (Kentish, et al., 2008). In the first stage, the eruption of the dispersed phase into

the water phase is obtained by creating interfacial waves through the acoustic field and in the second stage the pressure fluctuations, which are formed by the low frequency ultrasonic waves, cause the collapse of the vapor bubbles (Kentish, et al., 2008; Jafari, He, & Bhandari, 2006). So, the collapse of the vapor bubbles creates intense local turbulences and consequently causes a size reduction in oil droplets even to submicron sizes (Kentish, et al., 2008; Li & Fogler, 1978). However, due to high shear forces and cavitations, heat is generated through the process, which may be disadvantageous for cases where denaturation should be taken into consideration (McClements, 2005).

Many researches investigate the effects of ultrasonication in food emulsion preparation and there are many studies conducted on the comparison of ultrasonication with other homogenization methods. Kentish, et al. (2008) studied on the optimum working parameters of ultrasonication of flaxseed oil emulsions. Koh, et al. (2014) compared the effectiveness of ultrasonic homogenization with high shear homogenization and pressure homogenization on heat stability of whey protein solutions. Their findings show that, in whey protein solutions, ultrasonication is equally effective with other homogenization techniques in which shear is generated directly (Koh, et al., 2014). The reason for that is found to be the acoustic cavitation of ultrasonication (Koh, et al., 2014). According to the Abismail et al. (1999) and Maa & Hsu (1999), ultrasound emulsification is found to be more competitive than high-speed homogenization method considering droplet size and energetic effectiveness. Ertugay, Şengül, & Şengül (2004) examined the effect of ultrasonication on milk homogenization and found that ultrasonication is much more effective in milk homogenization than conventional homogenization method. According to Mongenot, Charrier, & Chalier's (2000) findings, ultrasound homogenization gives better efficiency and quality results in encapsulation of liquid cheese aroma when compared to ultra-turrax homogenization.

1.4.2.3 Microfluidization

Microfluidization is another homogenization technique frequently used in food industry and emulsion preparation. Although this method has been used for pharmaceutical industry for many years, its usage in food industry and specifically in flavor emulsions is rather recent (Jafari, He, & Bhandari, 2006). The device is known to create extremely small droplet sizes (McClements, 2005). A typical microfluidizer usually consists of three main parts; a fluid inlet, a pumping device and an interaction chamber where the fluids interact with each other after passing through channels (McClements, 2005). The process is based on dividing the flowing original stream into two streams, passing them through two separate channel with fine orifices and colliding them in the interaction chamber (Jafari, He, & Bhandari, 2006). With the help of high shear created by high pressure, the colliding streams form emulsions with extremely small droplet sizes (Jafari, He, & Bhandari, 2006). Besides the high shear, cavitation also helps to reduce the droplet size of the emulsion (Jafari, He, & Bhandari, 2006). Fig. 1.6 represents a typical microfluidizer.

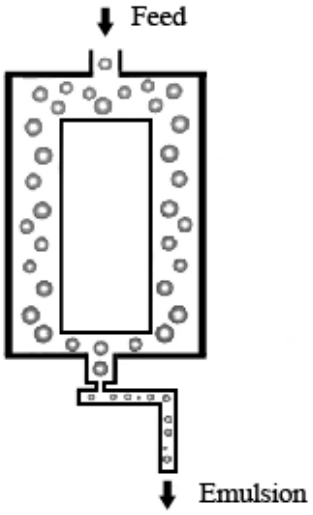

Figure 1.6 Microfluidizer

In the literature, there is an increasing number of researches investigating the different effects of microfluidization from other emulsion preparation methods. Many researcher agree that microfluidization gives better efficiency results than ultrasonication method (Abismail et al., 1999; Maa & Hsu, 1999; Jafari, He, & Bhandari, 2006; Jafari et al., 2008). However, some researchers oppose this opinion since microfluidization is found to be less practical due to high production costs, high equipment contamination and less aseptic processing (Tadros et al., 2004).

1.4.3 Drying Techniques

As mentioned above, there are different drying methods used for microencapsulation processes. Especially for the microencapsulation of essential oils, the most common drying techniques are spray drying and freeze drying (Jafari et al., 2008; Soottitantawat et al., 2003; Fernandes R. V., et al., 2013; Huynh et al., 2008; Akhtar & Dickinson, 2007).

1.4.3.1 Spray Drying

The first applications of spray drying dates back to 1800 in which the method was used for dairy products (Filkova, Huang, & Mujumdar, 2007). The first record of spray drying was mentioned in a patent in 1872 (Bhandari, Patel, & Chen, 2008). In 1930s this technique was used in encapsulation of flavors, which is why spray drying is regarded as one of the oldest processes for encapsulation technology (Fang & Bhandari, 2012).

Filkova, Huang, & Mujumdar (2007) defines spray drying as a suspended particle processing technique which uses the liquid atomization technique to create droplets which are than dried to individual particles while going through a hot

gaseous drying medium such as hot air. So, basically the method of spray drying consists of four steps; a) preparation of the feed solution, b) atomization, c) evaporation of the solvent and d) recovery of the microencapsulated powder. Especially for the application of spray drying in microencapsulation of food ingredients and nutraceuticals, the preparation of feed solution is more complex than conventional food materials since the feed solution has to be emulsified by previously explained methods (Fang & Bhandari, 2012). Atomization is considered as the most important step of the process since the type of the atomizer not only determines the drying time which changes the energy consumption but also the size distribution of the droplets, their trajectory and speed (Filkova, Huang, & Mujumdar, 2007). Thus, the drying efficiency, the powder properties, product quality and powder collection efficiency depend on the performance of the atomizer (Bhandari, Patel, & Chen, 2008). The contact of the liquid droplets with hot air is also important since it affects the evaporation rate of the solvent and so affects the efficiency of the process (Fang & Bhandari, 2012). The three basic air-droplet contact system are co-current contact, counter-current contact and mixed-flow contact where the names indicate the flow design of the feed and the air (Filkova, Huang, & Mujumdar, 2007). As Fang & Bhandari (2012) state, for the spray drying encapsulation of most food ingredients and nutraceuticals, co-current air-droplet contact system is employed. The common application of recovery of the powder is using cyclones, in which the powder is separated from the carrier air (Fang & Bhandari, 2012). The schematic diagram of a typical spray dryer is given in Fig. 1.7.

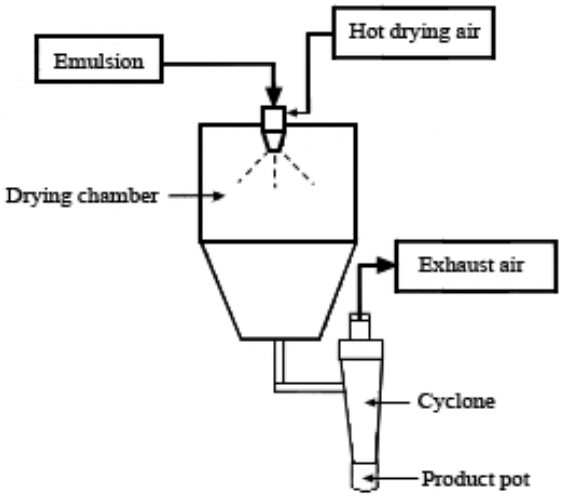

Figure 1.7 Spray Drier

As mentioned above, spray drying is one of the most common drying techniques used for the encapsulation of essential oils. Especially recently a lot of researchers investigated the effects of process conditions of spray drying on the efficiency and other quality parameters of encapsulated essential oils. Some examples of these essential oils are basil essential oil (Garcia, Tonon, & Hubinger, 2012), orange essential oil (Ascheri, Marquez, & Martucci, 2003-12), lime essential oil (Bringas-Lantigua, Valdes, & Pino, 2012) and oregano essential oil (Botrel, et al., 2012). Nevertheless, there are some limitations of spray drying process especially in encapsulation of food ingredients and nutraceuticals. Firstly, there are only a limited number of wall materials that could be used for spray drying encapsulation since almost all feed formulations are prepared in aqueous form (Gouin, 2004). Secondly, some spray dried products usually needs further application such as agglomeration since the droplet size decreases enormously (Fang & Bhandari, 2012). Another limitation of spray drying is that it cannot be used for some food samples, such as honeys, juices and other sugar-rich core materials since their low glass transition temperatures cause stickiness during the process (Bhandari, Datta,

& Howest, 1997). Spray drying is also not an appropriate drying technique for highly thermal sensitive materials since in the atomization step the feed meets with air with high temperature (Fang & Bhandari, 2012). Also, spray drying process has high installation costs (Filkova, Huang, & Mujumdar, 2007).

1.4.3.2 Freeze Drying

The origins of the freeze drying technique goes back to ancient Chinese and Peruvian Incas where people used to preserve their meats and potatoes by keeping them outside in cold winter which are then slowly dried by sublimation due to the low pressure of high altitudes. However, only for the last 80 years, freeze drying is considered as a part of science world (Hua, Liu, & Zhang, 2010).

Freeze drying, which can also be referred as lyophilization or cryodesiccation, is a drying process which is based on freezing the solvent or the suspension medium part of the product (usually water) and dehydrating the sample by the removal of the solvent fraction (Fang & Bhandari, 2012; Ratti, 2008). The removal of the solvent occurs in two ways; by sublimating the frozen solvent from the solid state to vapor state and by desorption of the unfrozen (sorbed) solvent again under low pressure (Liapis & Bruttini, 2007).

The process takes place in three main steps; a) the freezing step b) primary drying step and c) secondary drying step (Hua, Liu, & Zhang, 2010). In the case of encapsulation by freeze drying, a preliminary step of emulsification is applied to the sample, since the entrapment of the active agent in a glassy matrix of coating material is needed (Kaushik & Roos, 2008; Fang & Bhandari, 2012). The freezing step is the step where the complete solidification of the sample material occurs (Hua, Liu, & Zhang, 2010). This is a very important step, since the first separation between the phases (water part and the solute part) begins in this stage (Liapis & Bruttini, 2007). Also, the rate of solidification affects the texture of the frozen matrix and the morphological characteristics of the final product. Especially for

the encapsulation applications rapid cooling is found to result in more desired end-products (Fang & Bhandari, 2012). Primary drying or sublimation drying is the stage in which the frozen solvent (unbound solvent) is sublimated (Liapis & Bruttini, 2007). During this stage, low pressure is of great importance because the sublimation of free solvent requires pressures under the equilibrium vapor pressure of the solvent (Liapis & Bruttini, 2007). In the last stage of the drying, secondary drying, the remaining unfrozen (bound) solvent is removed from the sample. The drying rate in this stage is relatively low compared to the primary drying stage due to the porous structures of the materials (Ratti, 2008; Hua, Liu, & Zhang, 2010). The schematic diagram of a freeze dryer is given in Fig. 1.8.

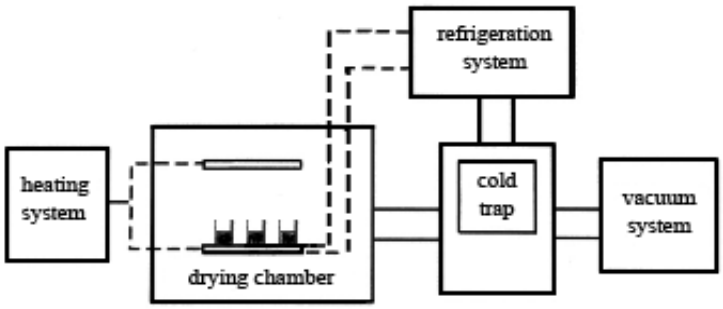

Figure 1.8 Freeze Drier

There are many applications of freeze drying technology both in food and pharmaceutical production. It is used in drying of non-living substances like blood plasma, hormones, enzymes, antibiotics; in drying of living substances like human cells, yeasts and microorganisms and in foodstuff like instant soups, coffee, milk powder and fruits and vegetables (Liapis & Bruttini, 2007; Hua, Liu, & Zhang, 2010; Fang & Bhandari, 2012). Also the utilization of lyophilization in the encapsulation of the food flavors is a recently emerging topic. As Fang & Bhandari (2012) mention, the freeze drying encapsulation has not been studied

much when compared to spray drying encapsulation of food flavors and there are only a limited number of studies in the literature. From those Ezhilarasiet al (2013) studied on freeze drying microencapsulation of Garcinia fruit extract, Tobitsuka, Miura, & Kobayashi (2006) studied the retention of pear aroma in freeze dried microcapsules, Kaasgaard & Keller (2010) investigated the coating material effect on freeze dried flavor oils and Lee et al. (2009) and Kaushik & Roos (2007) encapsulated D-limonene and limonene by freeze drying respectively.

Freeze drying is considered to give the highest quality food products among all drying methods (Liapis & Bruttini, 2007). It is a very advantageous technique especially in drying of heat sensitive compounds since the operation takes place at very low temperatures (Fang & Bhandari, 2012; Hua, Liu, & Zhang, 2010). It also keeps the color, the smell and the flavor of the food material effectively and prevents the surface hardening of the sample (Hua, Liu, & Zhang, 2010). However, the porous structure of the final product is one of the disadvantages of freeze drying applications since it will accelerate the rehydration and other chemical reactions when exposed to air (Fang & Bhandari, 2012; Hua, Liu, & Zhang, 2010). The requirements for freezing systems and vacuum systems and longer operational time also increase both the installation and operational costs of lyophilization compared to spray drying (Hua, Liu, & Zhang, 2010).

1.5 Objectives of the Study

In recent years, the alarming increase of diseases around the world push people towards consuming foods with supplementary nutrition. Encapsulation is one of the most efficient techniques to preserve the beneficial characteristics of the additional nutrients. By microencapsulation, the compound to be preserved is almost completely isolated from the external factors. By this technique, the unwanted taste and the odor of the ingredient is masked, the evaporation of the volatile components is prevented, the contact of the ingredient with oxygen is also

prevented by which the oxidation of lipids is decreased significantly, the release mechanism of the coated material is controlled better, the stability of the compound is increased and finally the shelf life of the desired compound is increased.

The benefits of the plant rosemary and its essential oil are better known day after day. In the literature, there are many studies on the antioxidant, antimicrobial, anticarcinogenic and many other effects of rosemary essence on health problems. Also, there are many investigations about the chemical composition of rosemary essential oil and its constituents. However, there is only a limited number of research on microencapsulation of rosemary essential oil. Employing freeze drying as the encapsulation technique and ultrasonication as the homogenization technique, and using whey protein isolate as the coating material were the novelties in this study.

The main objective of this study was to develop a different technique for microencapsulation of rosemary essential oil. Investigating the optimum coating material formulation for obtaining the highest drying and encapsulation efficiencies was another objective of this study. Moreover, the effects of different coating formulations on particle size and surface morphology of the capsules were analyzed. As the last part of the study, the storage stabilities of the encapsulated products were examined.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Rosemary (*Rosemarinus Officinalis* L.) essential oil, the core material used in the encapsulation process, was purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

Whey Protein Concentrate (WPC) containing 80% protein, was supplied by Tunçkaya Kimyevi Maddeler (Tuzla, İstanbul). The other coating material, maltodextrin (MD), was also supplied from Sigma Aldrich Chemical Co. (St. Louis, MO, USA) in two different dextrose equivalences (DE) (DE: 4.0-7.0 and DE: 13.0-17.0).

The Patcote 502K anti-foaming agent, which was used in the drying efficiency analyses, was supplied from Hydrite Chemical Company (WI, U.S.A.). In Soxhlet extraction, n-hexane was used as the solvent and it was purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

The chemicals magnesium chloride ($MgCl_2$), dipotassium phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4) and 1,8-cineole were also purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2 Preparation of Microcapsules

2.2.1 Preparation of Coating Materials

All the coating material solutions were prepared one day prior to the emulsification process.

The maltodextrin (MD) solutions were prepared in two different concentrations (10% and 30% by weight) for two different dextrose equivalences (DE: 4.0-7.0 and DE: 13.0-17.0). Distilled water was used to dissolve MD. The solutions were pre-mixed for 10 minutes by a magnetic stirrer (Heidolph MR 3001 K, Heidolph Instruments GmbH & Co, Schwabach, Germany) and then left in a shaking water bath (GFL 1086, Burgwedel, Germany) at 25^0C at 90 rpm for one night (18 hours) to obtain full hydration.

Whey protein concentrate (WPC) solutions were also prepared in two different concentrations (10% and 30% by weight). Phosphate buffer solution was used to dissolve WPC (Yazıcıoğlu, 2013). The preparation procedure of the phosphate buffer is adapted from Kuhlmann (2006). First, 1 M solutions of two stock solutions, dipotassium phosphate (K_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4), were prepared. Then, the mixture of two stock solutions were prepared in a ratio of 61.5 ml: 38.5 ml (K_2HPO_4: KH_2PO_4) in order to get a 100 ml of 1 M phosphate buffer. After that, solution was diluted until a 5mM (pH=7) solution was reached.

After necessary amounts of WPC were dissolved in phosphate buffer solution by using the magnetic stirrer, the solutions were left in the shaking water bath for one night (18 hours) at 25^0C to get full hydration.

2.2.2 Preparation of Emulsions

Prior to the addition of core material, the coating material solutions were weighed and mixed. From both coating material solution, whey protein solution and maltodextrin solution, 60 g were weighed from each in the same 250 ml glass beaker, which makes a 120 g of total coating material mixture. The mixture of coating materials were prepared in the following ratios: a) WPC: MD (DE: 4.0-7.0) = 1:3 b) WPC: MD (DE: 4.0-7.0) = 3:1 c) WPC: MD (DE: 13.0-17.0) = 1:3 and d) WPC: MD (DE: 13.0-17.0) = 3:1. The total soluble solid content of the mixtures were kept at 40% (w/w).

Rosemary essential oil was then added to the coating material mixtures in four different core-to-coating ratios: 1:80, 1:40, 1:20 and 1:10. To obtain these ratios 1.5 g, 3 g, 6 g, and 12 g of rosemary essential oil were added to 120 g of coating material mixtures respectively.

The emulsification process was performed in two stages of homogenization. First, pre emulsions were homogenized in high-speed homogenizer (IKA T25 digital Ultra-Turrax, Selangor, Malaysia) at 8000 rpm for 5 minutes. The speed of high-speed homogenizer was chosen according to the results of preliminary experiments. The second homogenization was done by using Ultrasonic Homogenizer (Sonic Ruptor 400, OMNI International the Homogenizer Company, Georgia, USA). Ultrasonic homogenizer was equipped with solid titanium 1" Solid and Tapped tip with a diameter of 25.4 mm and a length of 12.70 cm. The ultrasonication process was performed for 15 minutes at 40% power of 20 kHz using 50% pulse. These parameters were also based on the results of preliminary experiments. Especially during the ultrasonic homogenization the energy heats up the emulsions. To prevent excessive heating of the whey proteins in the emulsions, the beakers were placed in 4^0C water baths. In order to minimize the loss of the volatile oil due to splattering, the beakers were covered with plastic films during both homogenization steps.

The emulsions were frozen at -18^{0}C immediately after the homogenization procedure to prevent any coalescence or flocculation.

2.2.3 Freeze Drying

Fully frozen emulsions were dried in a freeze drier (Christ, Alpha 2-4 LD plus, Germany) for 48 hours at -50^{0}C and at 0.019 mbar. After lyophilization, dried samples were grinded into powder form with a glass rod. For SEM analysis, the capsules were further grinded with coffee grinder for 15 seconds.

2.3 Storage of the Microcapsules

The storage stability of the microencapsulated rosemary oil at 35.3 % ± 0.1 % relative humidity and at 15^{0}C was investigated. To obtain a relative humidity of 35.3 % ± 0.1%, saturated aqueous solution of magnesium chloride ($MgCl_2$) was prepared (Greenspan, 1976) and placed into the desiccators. Before the placement of the samples, the salt solution were kept overnight in the desiccators to reach equilibrium. The following samples were chosen as the samples for the storage stability experiments: WPC: MD (DE: 4.0-7.0) = 3:1 and WPC: MD (DE: 13.0-17.0) = 3:1 both with core-to-coating ratio of 1:20. In order to see the stability difference between the encapsulated rosemary oil and non-encapsulated rosemary oil, fresh essential oil was also stored in a desiccator for analyses. Two desiccators were used for the storage of two types of capsules and one desiccator was used to store the fresh oil. 5 g of specimen from each sample were taken at certain time intervals during 40 days of storage. The samples were then analyzed for their 1,8-cineole concentrations by GC-MS. 1,8-cineole is chosen as the reference compound since it is one of the major constituents of rosemary essential oil (Surburg & Panten, 2006).

2.4 Analysis of Emulsions and Microcapsules

2.3.1 Particle Size Analysis of Emulsions

Particle size distribution of six emulsions with different core-to-coating ratios and DE values were analyzed with Mastersizer 2000 (Malvern Instruments Limited, Worcestershire, UK). The chosen samples were WPC: MD (DE: 4.0-7.0) = 3:1 with 1:40, 1:20 and 1:10 core-to-coating ratios and WPC: MD (DE: 13.0-17.0) = 3:1 with 1:40, 1:20 and 1:10 core-to-coating ratios.

The mean particle size of the emulsions was represented with Sauter mean diameter, D32(μm), and was calculated with the following equation;

$$D32 = \frac{\sum n_i d_i^{\,3}}{\sum n_i d_i^{\,2}} \qquad (1)$$

where (d_i) represents the diameter of the particles in each size-class (μm) and (n_i) represents the number of particles in each size-class / unit volume of emulsion (McClements, 2005).

Span, the polydispersity of size distribution, measures the width of particles in dispersion and is calculated with the following formula (Karimi & Mohammadifar, 2014):

$$Span = \frac{[d(0.9) - d(0.1)]}{d(0.5)} \qquad (2)$$

where d(0.9), d(0.1), and d(0.5) are diameters at 90%, 10%, and 50% of cumulative volume respectively. In other words, [d(0.9) – d(0.1)] is the range of data and d(0.5) is the median diameter. During particle size analysis, the sonication was applied to the emulsions. However, during the analyses of emulsions which were prepared in order to see the effect of ultrasonication only, the sonication of Mastersizer 2000 (Malvern Instruments Limited, Worcestershire, UK) was switched off to have an accurate result.

2.3.2 Efficiency Analysis of Microcapsules

During freeze drying, especially in encapsulation of volatile components such as rosemary essential oil, a loss of the core material could occur (Jafari, He, & Bhandari, 2007). Thus, to determine the efficiency of the encapsulation process, two types of efficiency analyses were adopted: drying efficiency and encapsulation efficiency.

2.3.2.1 Drying Efficiency

The drying efficiency analysis was conducted to measure the remaining core material in the powder microcapsules. A hydrodistillation technique with Clevenger apparatus was chosen to measure the oil retention. Clevenger type of analysis is based on the idea of freeing both the encapsulated and non-encapsulated oil by boiling the microcapsules. Then, the total volatile oil is carried by the steam to the condenser to be read volumetrically (Atofani et al., 2010).

To measure the drying efficiency, first the total powder was weighed. Then, 10 g of powder was dissolved in 250 ml of distilled water in a 500 ml flask. In order to prevent foaming, one droplet of Patcote 502K anti-foaming agent (Hydrite Chemical Company, WI, U.S.A.) was added by a syringe and mixed with the

solution. The flask was then attached to the Clevenger apparatus. After 3 hours of distillation, the volume of the total oil was read from the volumetric arm of the Clevenger apparatus and multiplied with the density of rosemary essential oil (0.908 g/ml) to estimate the actual oil content in the capsules. The drying efficiency was then calculated according to the following equation (Jafari, He, & Bhandari, 2007):

$$\underset{Efficiency}{Drying}\ (\%) = \frac{[Oil\ \ content\ \ of\ \ microcapsules]}{[Oil\ \ content\ \ of\ \ emulsions]} \times 100 \qquad (3)$$

2.3.2.2 Encapsulation Efficiency

Encapsulation efficiency analysis aims to evaluate the ratio of surface oil to the entrapped oil in the capsule. Total oil content is determined by Clevenger apparatus. To measure the surface oil content, an analysis with Soxhlet apparatus was performed (Baranauskiené et al., 2007). Soxhlet type of analysis is based on the idea of dissolving only the non-encapsulated oil on the surface of the capsules in a solvent.

5 g of dried powder was weighed and trapped in filtration paper and washed for 3 hours with 250 ml of n-hexane in the Soxhlet apparatus. The excess hexane was then evaporated in a vacuum rotary evaporator (Heidolph Laborota 4000 efficient; Heidolph Instruments GmbH & Co, Schwabach, Germany) at 335 mbar and at 40^0C. The surface oil was then concentrated under nitrogen dryer (Turbovap LV Concentration Evaporator Workstation; Biotage, Charlotte, NC, USA). After the calculation of exact surface oil, the encapsulation efficiency was calculated using the following formula (Jafari, He, & Bhandari, 2007):

$$\underset{Efficiency}{Encapsulation}\ (\%) = \frac{[Total\ oil\ content - Surface\ oil\ content]}{[Total\ oil\ content]} \times 100 \qquad (4)$$

2.3.3 Surface Morphology Analysis of Microcapsules

To analyze the structures and the surface morphologies of microcapsules of rosemary essential oil, scanning electron microscope was used. Freeze dried samples were grinded for 15 seconds with coffee grinder (ARZUM AR151 Mulino Coffee Grinder, Turkey) to eliminate the structural differences inflicting from glass rod grinding. The samples were than coated with the mixture gold/palladium by HUMMLE VII Sputter Coating Device (ANATECH, Union city, CA, USA). The scanning electron microscopy (SEM) (JSM-6400 Electron Microscope, Jeol Ltd., Tokyo, Japan) was equipped with NORAN System 6 X-ray Microanalysis System and Semafore Digitizer. The images of the microcapsules were taken at three different magnifications; 50x, 500x and 5000x.

2.2.4 GC-MS Analysis

The stored microcapsules and fresh rosemary oil were analyzed for their 1, 8-cineole content. The oil was extracted from the powder by Clevenger apparatus and then analyzed with Gas chromatography/Mass spectrometry (GC-MS) system (Agilent Technologies 6890N Network GC System coupled to Agilent Technologies 5973 Network Mass Selective Detector, USA). Quantitative analyses could be done using FID with a capillary column (Agilent 19091s-433 HP-5MS with a 5% phenyl methyl siloxane stationary phase and with a size of 30m x 0.25mm x 0.25μm). An Agilent Tecnologies 7683B Series Injector (Thailand) was used to inject the sample. The data were analyzed by MSD ChemStation software program and helium was used as the carrier gas.

The following GC-MS conditions were used during the analyses: split ratio 16.5:1; injection volume 1 µL; oven temperature program, holding at 40°C for 1 min, rising to 180°C with 4°C/min with a total run time 39 min; MSD transfer line temperature, 230°C; MSD quadrupole temperature, 150°C. Solvent delay was for 4.0 min. The GC analysis was performed with the following conditions: H_2 flow rate, 35 mL/min; air flow rate, 400 mL/min; make-up flow rate of 48.8 mL/ min with a make-up gas type, He; FID temperature, 275°C.

Prior to injection, the samples were diluted in n-hexane with a ratio of 1:100 (µl:µl). The calibration curve for 1,8-cineole was prepared with five different concentrations in ml/ml (1/50, 1/100, 1/200, 1/400, 1/800 ml/ml) with n-hexane. Correction of variation values (R^2) of all calibration curves were obtained as greater than 0.99.

2.2.5 Statistical Analysis

To understand if there was a significant difference between the samples, the results were analyzed by analysis of variance (ANOVA) ($p \leq 0.05$). When a significant difference was found between the samples, Duncan's Multiple Comparison Test was applied ($p \leq 0.05$) by using SAS software version 9.1 (SAS Institute Inc., NC, USA).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Drying Efficiency

Drying methods are as important as the formulation of coating materials and the ratio of core and coating materials for the efficiency of microencapsulation processes. This is because especially in the encapsulation of volatile materials, a great amount of core material could be lost during the drying process. That is why, for obtaining the optimum formulation for the encapsulation of rosemary essential oil, first, drying efficiencies of different formulations are compared in this study.

Fig. 3.1 represents the drying efficiencies of microcapsules encapsulated with coating containing WP and MD at a ratio of 3:1, using MD having DE values of 13-17 and 4-7 and with different core to coating ratios. The results show that, regardless of the value of dextrose equivalences, core to coating ratios of 1:80, 1:40 and 1:20 showed no significant difference ($p>0.05$) in drying efficiencies (Table A.1). However, the drying efficiency was significantly lower when core to coating ratio was 1:10 ($p\leq0.05$) (Table A.1). This difference could be explained by the oil load of the emulsions. As the amount of oil used in the preparation of the emulsions increased, the solid content in the mixture became insufficient to cover and entrap the excessive amount of oil. Therefore, more non-entrapped volatile oil was lost under the vacuum of freeze drying. Although the oil load was also increased when core to coating ratio was increased from 1:80 to 1:40 or 1:20, the drying efficiencies showed no significant difference. Based on this result, in the choice of optimum formulation, the core to coating ratio of 1:80 was

eliminated for the rest of this study since the amount of oil encapsulated in that formulation was too low.

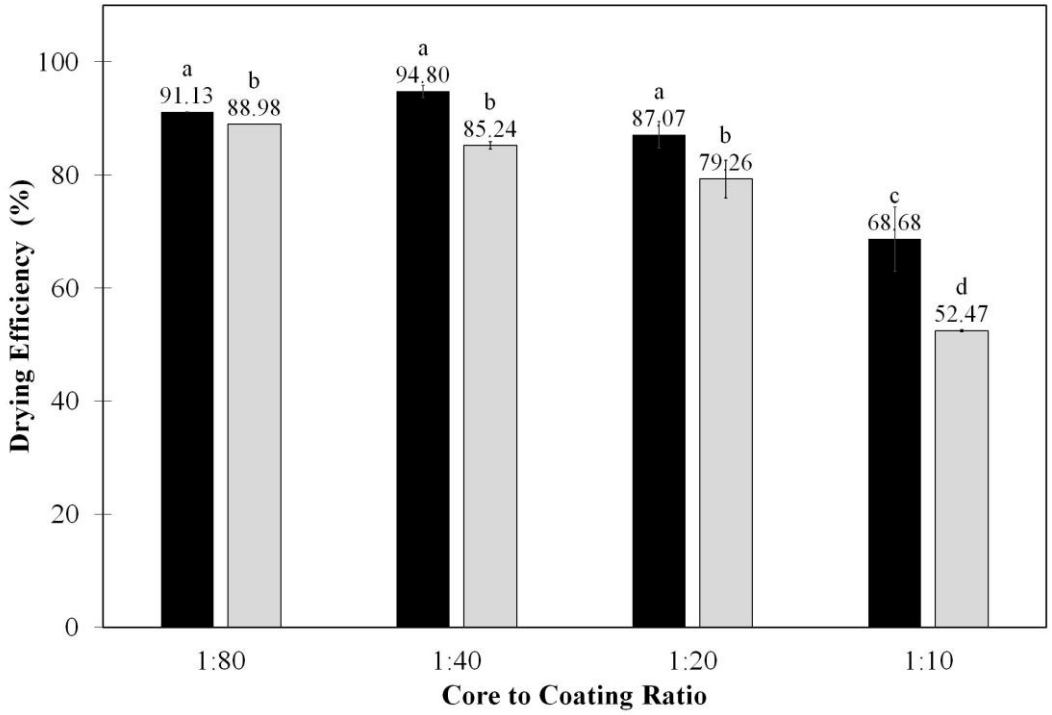

Figure 3.1 Drying efficiencies of microcapsules encapsulated with WP and MD at a ratio of 3:1, different core to coating ratios and maltodextrins having different DE values ; (■): DE:13-17, (■): DE:4-7. Different letters represent significant difference ($p \leq 0.05$)

The drying efficiency differences when maltodextrins having different DE values were used in encapsulation, could be clearly seen in Fig. 3.1. According to the results, the formulation with the maltodextrin with DE:13-17 gave higher drying efficiency than the capsules prepared with maltodextrin with DE:4-7. This indicated that as DE value increased, the retention of the volatiles in the wall

matrix increased. The results are in accordance with the findings of Shah et al., (2012). In the encapsulation of thymol with a wall mixture of whey protein isolate and maltodextrin, a change in DE value from 4 to 10 and 18 increased thymol retention ratios from 37.3% to 50.9% and 63.4%, respectively. The similar findings of Sheu & Rosenberg (1998) also supports this results with an explanation of molecular weight ratio of the wall matrix. According to Sheu & Rosenberg (1998), as DE value increased the proportion of low molecular weight carbohydrates increased. This provided less disrupted capsules during drying which resulted in higher drying efficiencies. Also as particles get smaller, the drying rates increased, which enhanced the solidification rate and also retention of volatiles.

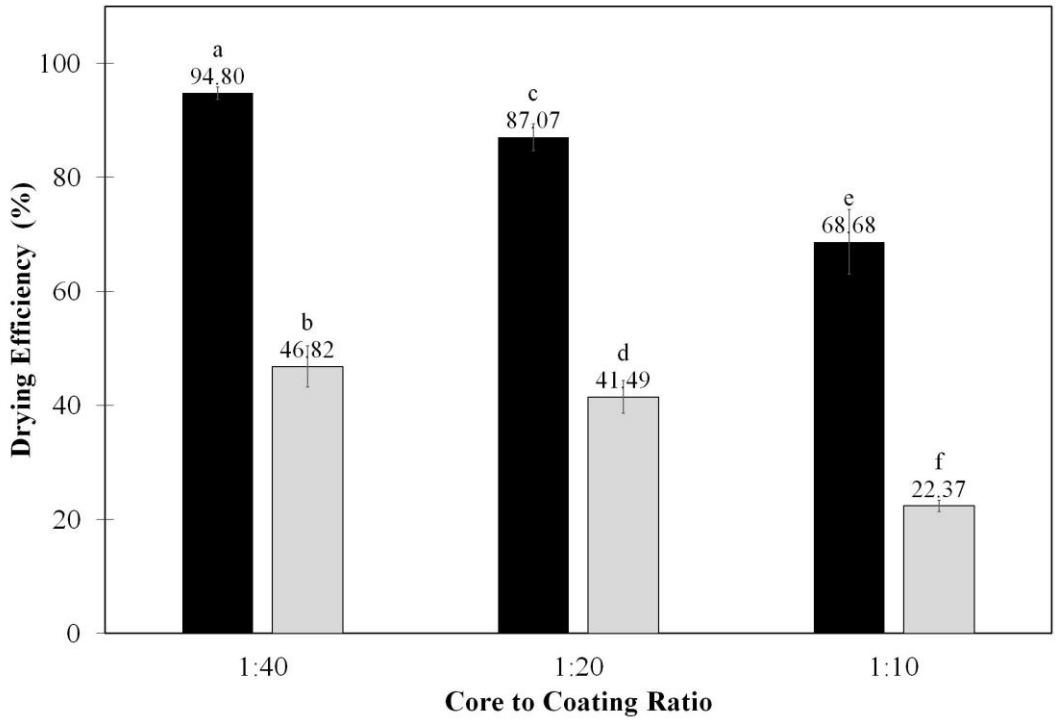

Figure 3.2 Drying efficiencies of microcapsules encapsulated by maltodextrin with DE:13-17 at different core to coating ratios and different WP:MD ratios; (■): 3:1 and (■): 1:3. Different letters represent significant difference ($p \leq 0.05$)

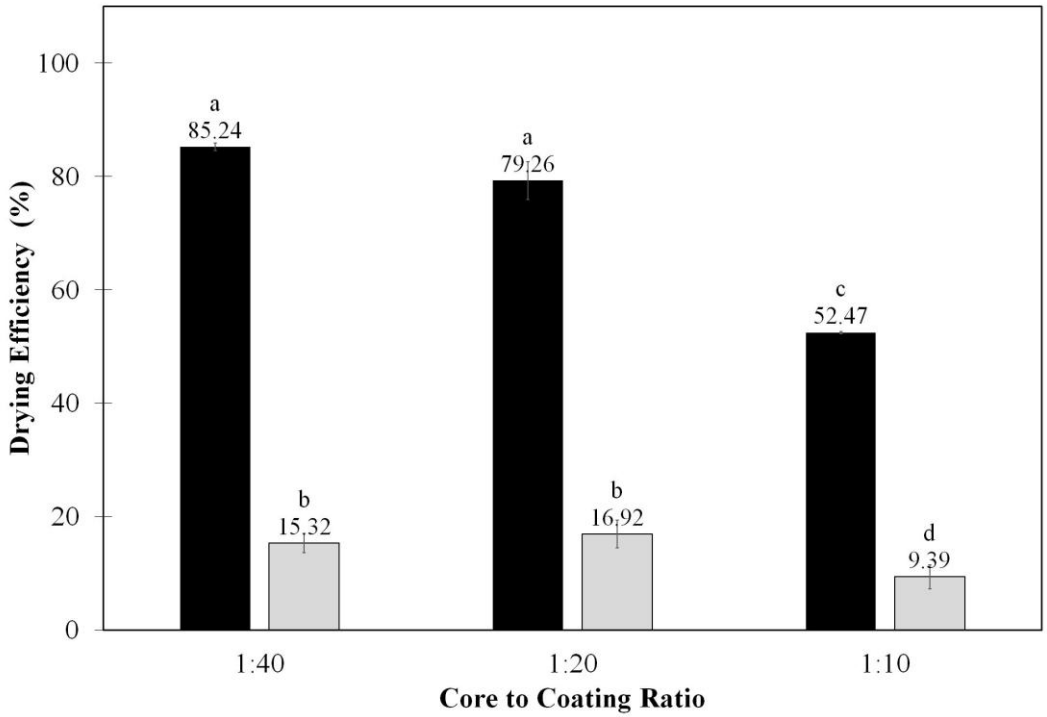

Figure 3.3 Drying efficiencies of microcapsules encapsulated by maltodextrin with DE:4-7 at different core to coating ratios and different WP:MD ratios; (■): 3:1 and (■): 1:3. Different letters represent significant difference ($p \leq 0.05$)

Effect of concentrations of whey protein concentrate and maltodextrin in coating formulations with different core to coating ratios on drying efficiency values can be seen in Fig. 3.2 and Fig. 3.3.

From Fig. 3.2, it can be understood that for MD having DE value of 13-17 and for all core to coating ratios (1:40, 1:20 and 1:10) drying efficiency decreased noticeably as WP:MD ratio changed from 3:1 to 1:3. The decrease in drying efficiency as concentration of WP decreased in coating formulation was also significant when MD having DE value of 4-7 was used (Fig. 3.3, Table A.3) ($p \leq 0.05$). Sheu & Rosenberg (1998) explained the positive effect of increasing

concentration of whey proteins on drying efficiency by their contribution to surface morphology of the capsules. According to that, whey proteins have positive effects on both drying rate and mechanical properties of the wall matrix, which decreases the number of surface cracks on the capsules and therefore increases the drying efficiency.

According to the results of drying efficiencies for WP:MD ratios, WP:MD ratio of 1:3 is not a reasonable choice for the optimization of the encapsulation formulation.

3.2 Encapsulation Efficiency

Encapsulation efficiency is probably the most important criteria for an encapsulation process to be considered as successful. To obtain the optimum formulation for encapsulation of rosemary essential oil, the encapsulation efficiencies of different formulations, which were discussed in drying efficiencies section, are compared as well.

The effects of WP:MD ratios, DE values of MD used in wall material and core to coating ratios on the encapsulation efficiencies of the microcapsules are illustrated in Fig. 3.4 and Fig. 3.5.

As can be seen in Fig. 3.4, when MD having DE value of 13-17 was used in wall material, the increase in WP concentrate in the coating formulation increased encapsulation efficiency values significantly for all core to coating ratios ($p \leq 0.05$) (Table A.4). According to the statistical analyses, the capsules prepared by maltodextrin with DE:4-7 also showed similar results ($p \leq 0.05$) (Fig. 3.5, Table A.5).

One of the reasons of this trend is the good emulsification properties of whey proteins (Jafari, et al., 2008). In addition, maltodextrins lack surface-active properties and this makes them poor wall materials when they are used alone for

encapsulation processes especially when they are used for encapsulation of volatile core materials (Sheu & Rosenberg, 1998). Another reason for the effectiveness of whey proteins on encapsulation efficiency is that the unfolding and adsorption on the oil-water interfaces change protein structures, which later causes the formation of a resistant and stable layer over the oil droplets. According to Sheu & Rosenberg (1995), a minimum amount of whey protein/carbohydrate ratio of 1:19 is required to make a stable encapsulation. Thus, the increase in whey protein concentration results in higher encapsulation efficiencies.

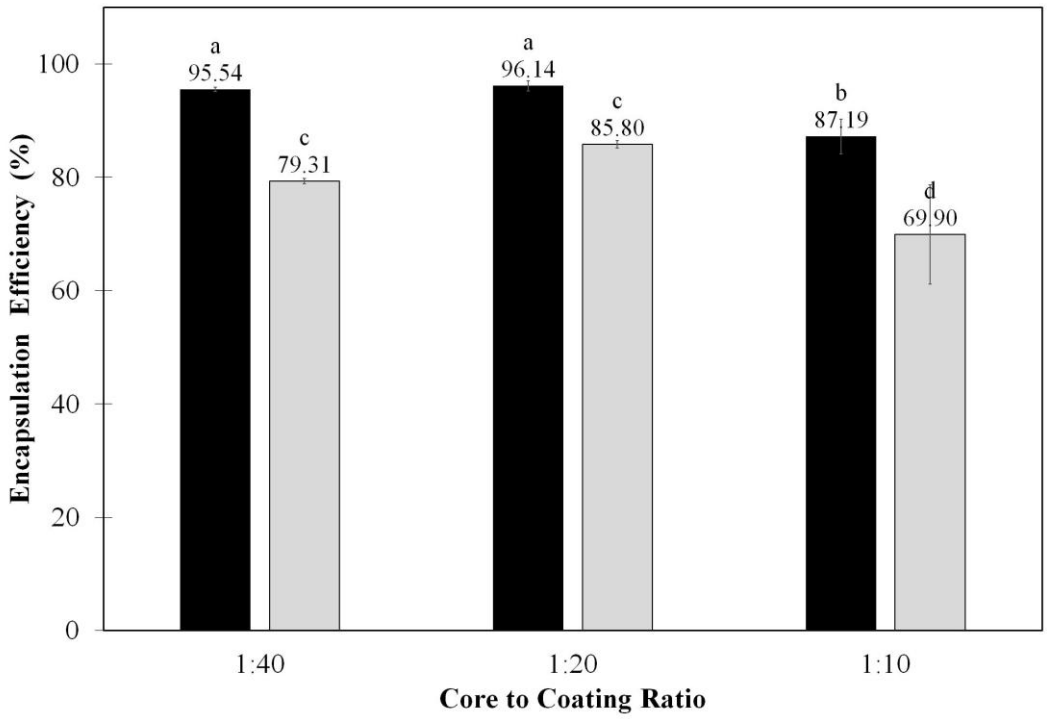

Figure 3.4 Encapsulation efficiencies of microcapsules encapsulated by maltodextrin with DE:13-17 at different core to coating ratios and different WP:MD ratios; (■): 3:1 and (■): 1:3. Different letters represent significant difference ($p \leq 0.05$)

The decrease in encapsulation efficiencies as WP:MD ratio decreases can also be explained by the reduction in viscosity of the wall material solutions (Jafari, et al., 2008). Although the solid content ratio remained the same when WP:MD ratio was changed from 3:1 to 1:3, the amount of larger particles (whey proteins) decreased which caused a decrease in the viscosity of the coating solution. Thus, as the viscosity of the wall material solution decreased, the encapsulation efficiency values also decreased. The findings of Rosenberg & Sheu (1996) supported these results, since they found an increasing trend of encapsulation efficiencies as the whey protein concentration increased from 10% to 30% in the encapsulation of both ethyl butyrate and ethyl caprylate.

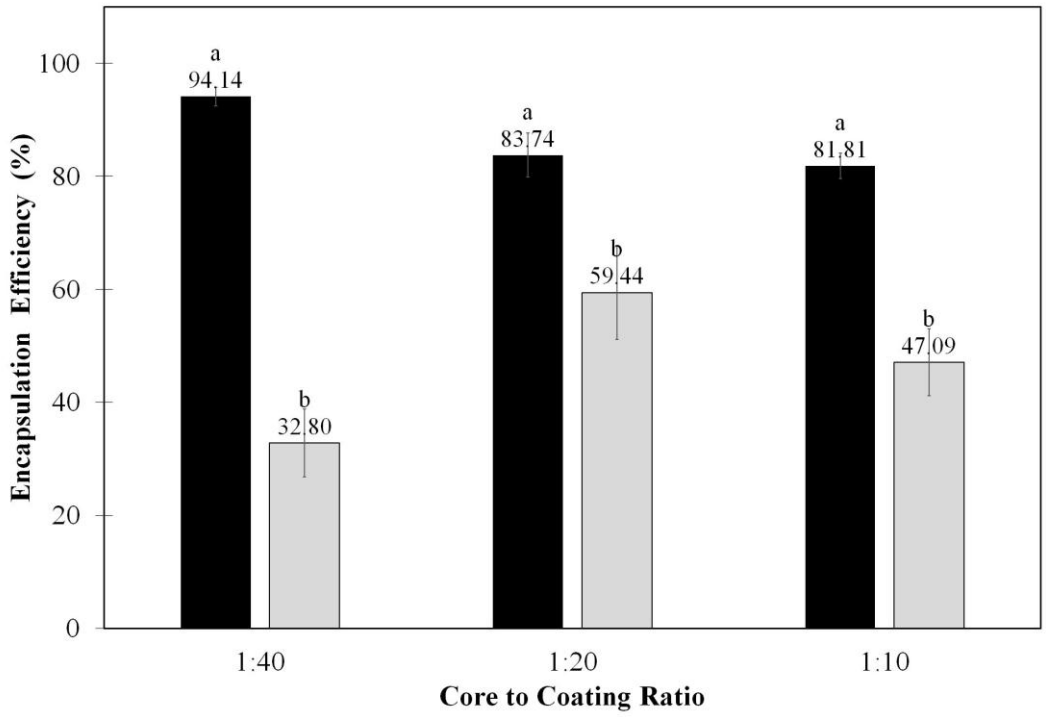

Figure 3.5 Encapsulation efficiencies of microcapsules encapsulated by maltodextrin with DE:4-7 at different core to coating ratios and different WP:MD ratios; (■): 3:1 and (■): 1:3. Different letters represent significant difference ($p \leq 0.05$).

As mentioned in the previous section, since both the drying and encapsulation efficiency values when WP:MD ratio of 1:3 was used as a coating material were significantly insufficient when compared to WP:MD ratio of 3:1, this encapsulation formulation was omitted from the formulation for further analyses.

The oil content of the emulsions and the maltodextrin types are also two important factors considered in encapsulation efficiency analyses. Fig. 3.6 represents the results of encapsulation efficiency values of microcapsules encapsulated with WP:MD ratio of 3:1 for MD having DE value of 13-17 and 4-7, and for three different core to coating ratios 1:40, 1:20 and 1.10.

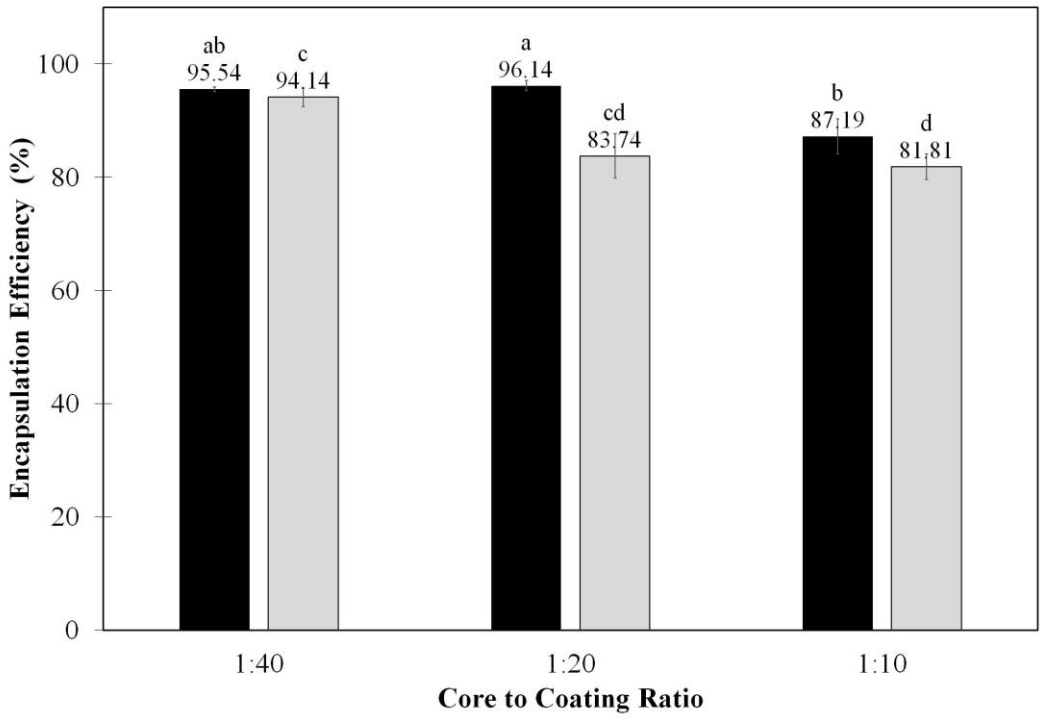

Figure 3.6 Encapsulation efficiencies of microcapsules encapsulated with WP and MD at a ratio of 3:1, different core to coating ratios and maltodextrins having different DE values; (■): DE:13-17, (■): DE:4-7. Different letters represent significant difference ($p \leq 0.05$)

When only the difference between maltodextrin types was considered, it was seen that the encapsulation efficiencies of the capsules prepared with MD having different DE values were significantly different from each other ($p \leq 0.05$) (Fig. 3.6, Table A.6). It is seen that for all core to coating ratios, the formulation with MD having DE:13-17 gave higher encapsulation efficiency results than formulation with DE:4-7. This result was also in correlation with the drying efficiencies of the same formulations (Fig. 3.1). The results indicated that maltodextrin with higher DE value helped to entrap more oil inside of the capsule and left less of the total oil on the surface of the capsules. The detailed results of surface oil contents of the formulations can be seen more clearly in Table 3.1. Higher surface oil content results also implied that the formulation with DE of 4-7 had poorer encapsulating properties. In the literature, there are many studies which are in consistency with these results. For instance, the findings of Sheu & Rosenberg (1995) showed that, at different WP/carbohydrate ratios, the encapsulation efficiency results of ethyl caprylate increased as the DE value of maltodextrin was changed from 5 to 24. According to Jafari et al., (2008), the positive effect of increasing DE value was due to the decreasing permeability of capsule to oxygen. The findings of Hogan et al., (2001) about the encapsulation of soya oil also indicated that as the amount of smaller oligosaccharides increased (higher DE value), the porosity of the capsules decreased which resulted in higher encapsulation efficiencies.

Table 3.1 Surface oil content (g oil/100 g capsule) of microcapsules prepared with MD having DE values at different WP:MD and core to coating ratios.

DE Values	WP:MD	Core:Coating	Surface Oil Content (g oil/ 100 g capsule)
13-17	3:1	1:40	$0.49\pm0.044^{d*}$
13-17	3:1	1:20	0.64 ± 0.130^{d}
13-17	3:1	1:10	2.69 ± 0.474^{ab}
13-17	1:3	1:40	1.22 ± 0.121^{dc}
13-17	1:3	1:20	1.39 ± 0.164^{dc}
13-17	1:3	1:10	3.11 ± 0.772^{a}
4-7	3:1	1:40	0.59 ± 0.164^{d}
4-7	3:1	1:20	2.40 ± 0.711^{bc}
4-7	3:1	1:10	3.85 ± 0.236^{a}
4-7	1:3	1:40	$1,36\pm0.030^{dc}$
4-7	1:3	1:20	1.47 ± 0.304^{dc}
4-7	1:3	1:10	2.35 ± 0.211^{bc}

*Different letters represent significant difference ($p \leq 0.05$)

Another parameter that Fig. 3.6 represents about the encapsulation efficiencies of microcapsules is the core to coating ratios of the emulsions. For both DE values of MD (13-17 and 4-7) encapsulation efficiency values of capsules prepared with core to coating ratio of 1:40 and 1:20 were not significantly different ($p>0.05$)

(Table A.6). Similarly, the change of core to coating ratio from 1:20 to 1:10 did not cause any significant difference on efficiency values of either DE values ($p>0.05$) (Table A.6). Even though there are no significant differences between the paired comparisons, when the overall results are compared, it can be seen that the encapsulation efficiencies decreased with the increasing oil content. As the oil load increased from 1:40 to 1:10 in the emulsion, the efficiencies decreased from 95.54% to 87.19% and 94.14% to 81.81% for formulations with MD having DE:13-17 and DE:4-7, respectively. The result indicated that as the oil amount increased, it became harder for solid particles to cover the droplets and more oil was left on the surface of the capsules (Table 3.1). This situation was consistent with other investigations. Such as, in the study of encapsulation of soya oil with WPC, as the oil/protein ratio increased from 0.25 to 3.0, the microencapsulation efficiency decreased from 50-60% to 2-10% (Hogan et al., 2001). Hence briefly, the reason of this inverse relation between the oil amount and encapsulation efficiencies was the insufficiency of the solid materials to produce a strong structural layer around the oil droplets.

Based on encapsulation efficiency results, in the choice of the optimum formulation, it might be considered that there were no differences between the encapsulation powers of sequential paired comparisons of core to coating ratios. However, when the oil loads were considered, the amount of oil was twice as much in 1:20 than in 1:40. On the other hand, as mentioned above there was a significant difference between 1:40 and 1:10. That is why, for surface morphology analyses and storage stability analyses, the formulations of WP:MD ratio 3:1, core to coating ratio 1:20 for both DE values were chosen.

3.3 Particle Size

Particle size analysis has been performed in different emulsion formulations, before the drying process. Table 3.2 shows the particle size distributions, span values and the specific surface area (SSA) values of the emulsions prepared with

maltodextrins having different dextrose equivalent values (DE:13-17, DE:4-7), different core to coating ratios (1:40, 1:20 and 1:10) with a WP:MD ratio of 3:1. The particle size distributions of the emulsions were analyzed in terms of Sauter mean diameter (D32(μm)).

As can be seen from Table 3.2, a change in DE values did not have any effect on particle sizes of the emulsions. According to the statistical analyses, there was no significant difference between the D32(μm) values of emulsions prepared with DE:13-17 and DE:4-7 in all core to coating ratios (1:40, 1:20 and 1:10) ($p>0.05$) (Table A.7). From this results, it can be inferred that the differences between the drying and encapsulation efficiencies of capsules prepared with MD having different DE values were not the consequences of different particle sizes of the emulsions. These results were also in accordance with the findings of Hogan et al., (2001), who found no significant difference between the D4,3 (mm) values of the soya oil emulsions prepared with six different dextrose equivalences ranging from DE:5.5 to DE:50.

Another factor that Table 3.2 showed was the effect of ratio of core materials to the wall materials on particle size of emulsions. The effect of different core to coating ratios can also be seen for DE values of 13-17 and 4-7 in Fig. 3.7 and Fig. 3.8, respectively.

Table 3.2 Particle size analyses of emulsions prepared with MD having different DE values and different core to coating ratios with WP: MD ratio of 3:1

DE values	Core:Coating	D32(μm)	Span	SSA (m^2/g)
4-7	1:40	0.187^{c}*	16.769^{a}	32.1^{a}
4-7	1:20	0.207^{b}	5.295^{ab}	29.0^{b}
4-7	1:10	0.255^{a}	3.708^{b}	23.5^{c}
13-17	1:40	0.188^{c}	20.813^{a}	31.9^{a}
13-17	1:20	0.205^{b}	12.423^{ab}	29.2^{b}
13-17	1:10	0.246^{a}	4.238^{b}	24.4^{c}

*Different letters represent significant difference ($p \leq 0.05$). SSA: Specific surface area

As can be seen in Fig. 3.7, for the emulsions prepared with MD having DE:4-7, as core to coating ratio changed from 1:40 to 1:20 and 1:10, the particle size distribution curve shifted slightly to larger particle size side of the graph. This means that as the oil concentration increased in the emulsion, the particle size of the emulsion became larger. The Sauter mean diameter values gave the same outcome as well. As the core to coating ratio increased from 1:40 to 1:20 and 1:10, the D32 values also increased from 0.187 μm to 0.207 μm and 0.255 μm, respectively. Fig. 3.8 and Table 3.2 also implied the same trend for emulsions with MD having DE value of 13-17. For the core to coating ratios of 1:40, 1:20 and 1:10, the D32 values were 0.188 μm, 0.205 μm and 0.246 μm respectively.

Hogan et al., (2001) and Taneja et al., (2013) also reported similar results, in which they found that particle sizes increased with increasing core/wall ratios. This phenomenon could be related to coalescence. Because, as oil concentration increased, the protein amount became insufficient for the adsorption at the core/wall interfaces which led to coalescence and an increase in the droplet size in the emulsion.

Additionally, the span values and the SSA values of the emulsions changed with the increasing oil ratio as expected (Table 3.2). For both DE values, span values decreased as the oil content of the emulsions increased. Fig. 3.7 and Fig. 3.8 showed that, even though the number of particles with small sizes (<1 μm) increased with the decreasing oil concentration, the curves became wider. This can be explained by the increasing inhomogeneity in the emulsions. Such that, as the oil amount was reduced, more and more protein was freed from the oil droplets, which later coagulated and formed larger protein clusters. The inverse proportion of SSA values with core to coating ratios were also expected since increasing particle sizes caused a decrease in SSA values.

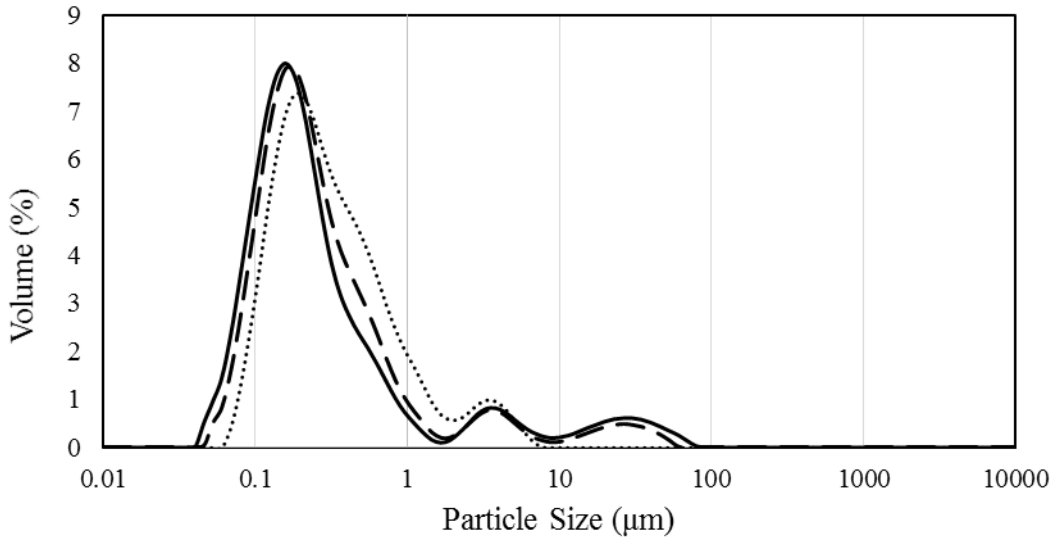

Figure 3.7 Particle size distributions of emulsions prepared with WP:MD ratio of 3:1, maltodextrin with DE:4-7 and different core to coating ratios; 1:40 (solid line), 1:20 (dashed line) and 1:10 (dotted line).

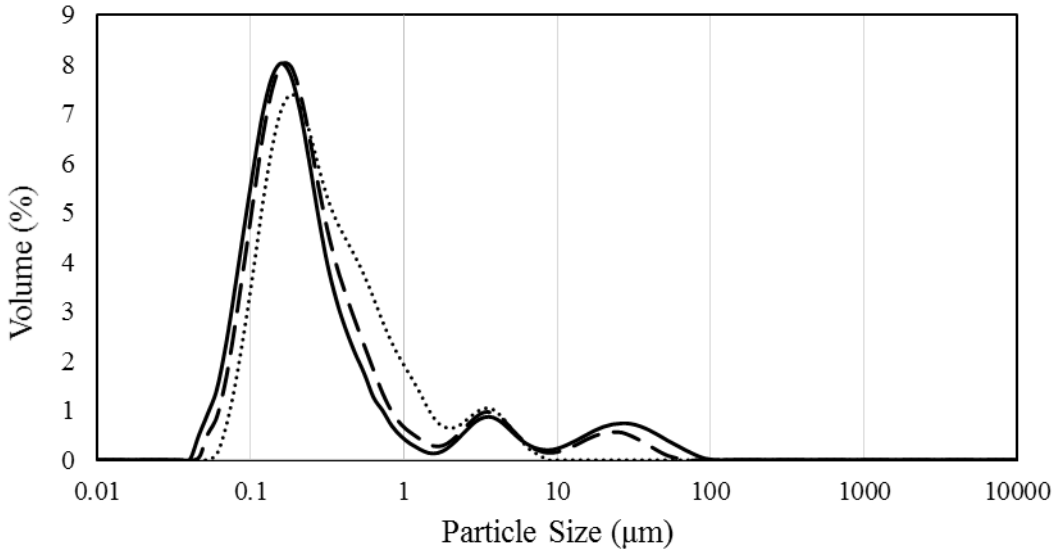

Figure 3.8 Particle size distributions of emulsions prepared with WP:MD ratio of 3:1, maltodextrin with DE:13-17 and different core to coating ratios; 1:40 (solid line), 1:20 (dashed line) and 1:10 (dotted line).

In order to see the effect of the ultrasonication technique on the particle size distribution of the emulsion, sample with DE:13-17, WP:MD ratio of 3:1 and core to coating ratio of 1:20 was analyzed before and after the ultrasonication step of homogenization. In Table 3.3, the particle size values are given for sample with and without ultrasonication (just Ultra-turrax homogenization). Table 3.3 clearly showed that ultrasonication had a significant effect on decreasing the particle size. Also it can be seen from Fig. 3.9 that while the majority of the particles were in a range between 0.1-1 μm for ultrasonicated sample, for the sample which was homogenized only with Ultra-turrax mixer the range was between 0.5-10 μm. The span of the particle size distribution curves and the SSA values were also correlated with decreasing particle size values (Table 3.3). Based on these results, it can be said that ultrasonication has a positive impact on emulsification. This effect is proven by many researchers in the literature. For instance, Jafari, He, & Bhandari (2007c) compared Silverson high speed blender with ultrasonication in the encapsulation of d-limonene and concluded that ultrasonication was a better emulsification method than homogenization with a usual high speed mixer. The reason of this was simply the energy supplied to the solution during homogenization. In the methods with high speed blenders, the energy given to the solution was low when compared to ultrasonication. As known, as energy density increases the droplet becomes smaller (Jafari, He, & Bhandari, 2007b). Another reason of this performance differences of two homogenizers was the acting forces. In high-speed mixers, the active forces are the shear stresses, which are not sufficient enough for droplet disruption. However, in ultrasonication method, the active force is cavitation, which are better at droplet disruption than high-speed mixers.

Table 3.3 The effect of homogenization technique on the particle size results of the emulsion prepared by maltodextrin with DE:13-17 and core to coating ratio of 1:20

Homogenization Technique	D32(μm)	Span	Specific Surface Area (m^2/g)
Ultraturrax	1.428	13.039	4.2
Ultraturrax + Ultrasonic	0.254	26.948	23.6

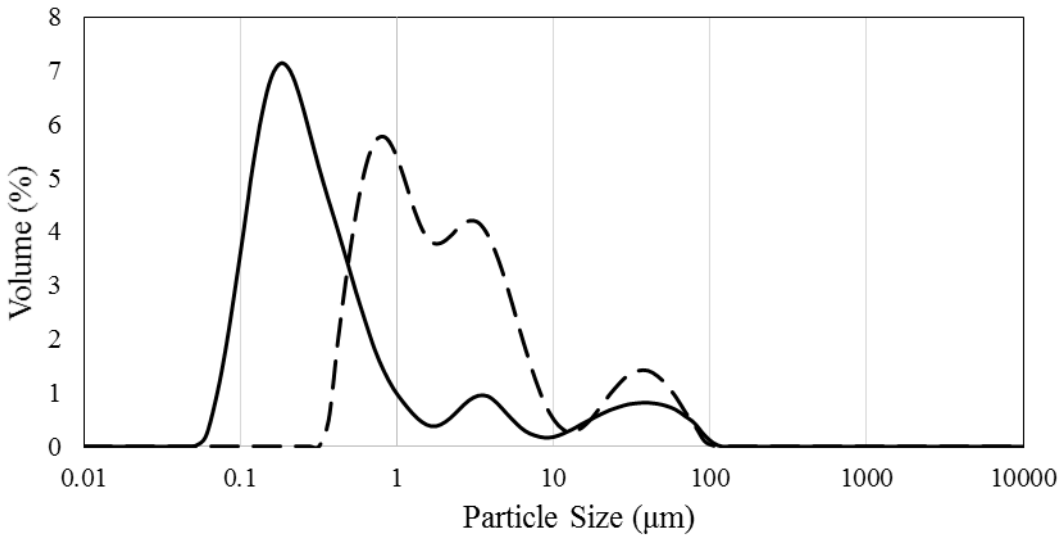

Figure 3.9 Particle size distributions of emulsions prepared with ultrasonic homogenization (solid line) and Ultra-turrax homogenization (dashed line) with WP:MD ratio of 3:1, maltodextrin with DE:13-17 and core to coating ratio of 1:20.

3.4 Surface Morphology of Microcapsules

In order to examine the surface morphology of microcapsules and to see the difference between the sizes and the structures of the capsules, SEM analysis was conducted. From different core to coating ratios and WP:MD ratios, samples having the highest encapsulation efficiencies, that is the samples having core to coating ratio of 1:20 and WP:MD ratio of 3:1, were analyzed. To understand the effects of DE values on the morphologies of capsules, microcapsules prepared by maltodextrin with DE:13-17 and with DE:4-7 were analyzed. The samples were investigated under three different magnifications.

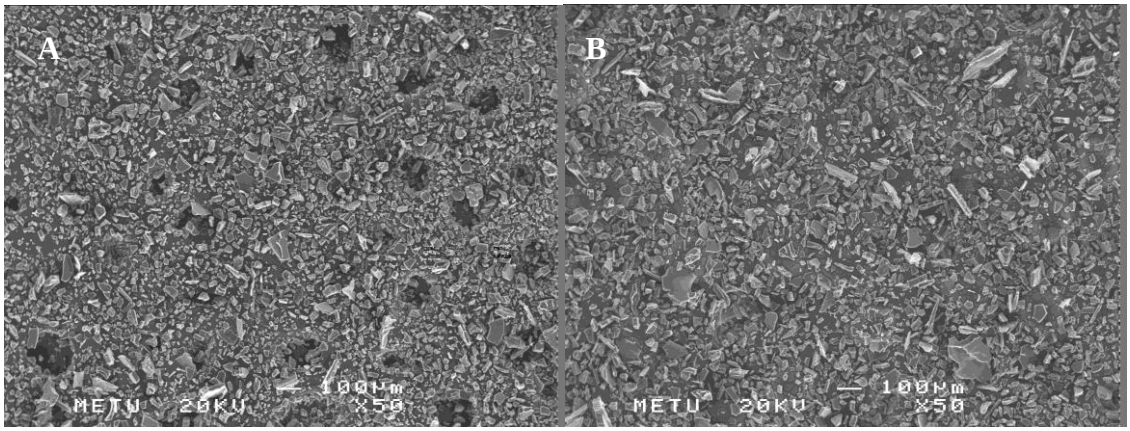

Figure 3.10 Scanning Electron Microscope images (×50 magnification) of microcapsules having WP:MD ratio of 3:1 and core to coating ratio of 1:20 prepared by MD having different DE values; (A): DE:13-17, (B): DE:4-7

The SEM images of the samples with ×50 magnification are illustrated in Fig. 3.10. From the images it can be seen that both sample with DE:13-17 (Fig. 3.10A) and sample with DE:4-7 (Fig. 3.10B) had almost evenly distributed particles throughout the images. This distribution was gained through the grinding of the

particles for 15 seconds using coffee grinder in order to eliminate the deficiencies born from grinding manually with glass rod.

As can be seen from Fig. 3.10, there was no difference between the sizes of the particles of two samples prepared with maltodextrins having different DE values. This result was also in accordance with the particle size analysis of emulsions, in which there is no significant difference between D32 (μm) values of the emulsions (Table 3.2). Thus, it could be concluded that DE value had no effect on particle size of powders. This result was in correlation with the findings of Hogan, et al., (2001). Hogan, et al., (2001) investigated the effect of DE value on the particle size of the microcapsules of soya oil. They also found that there were no significant difference between the powder particle sizes of the capsules prepared by carbohydrates with DE values of 5.5, 14, 18.5, 28 and 38.

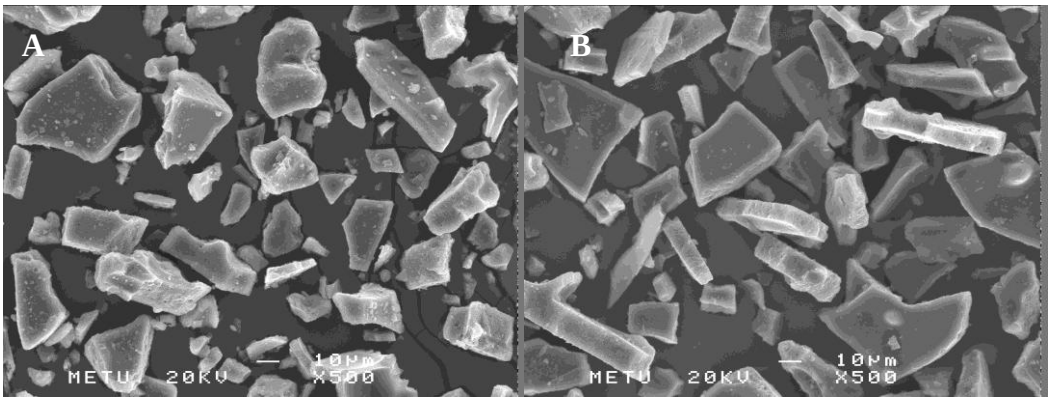

Figure 3.11 Scanning Electron Microscope images (×500 magnification) of microcapsules having WP:MD ratio of 3:1 and core to coating ratio of 1:20 prepared by MD having different DE values; (A): DE:13-17, (B): DE:4-7

The images of powders with the formulation WP:MD ratio of 3:1 and core to coating ratio 1:20 are represented in Fig. 3.11 with ×500 magnification. As could

be seen from the figure, capsules had irregular shapes and sharp edges. The structures resemble flakes or broken glass pieces. Anandharamakrishan, Rielly, & Stapley (2010) explained this difference between freeze dried sample structure and structures of other common drying methods by sudden sublimation in freeze drying. The ice crystals, formed during the freezing step of freeze drying, sublimate suddenly and leave irregularities and porous structures on the microcapsules.

The images with ×500 magnification gave clear images of surface morphologies of microcapsules prepared with MD having DE:13-17 (Fig. 3.11A) and microcapsules prepared with MD having DE:4-7 (Fig. 3.11B). It can be seen that both samples had smooth surfaces free of cracks and dents. This lack of surface deformations could be explained by the high content of whey protein concentrate of wall matrices. Sheu & Rosenberg (1998) also proved this theory in the comparison of surface morphologies of ethyl caprylate microcapsules. They also found that the presence of surface dents and cracks were inversely related to the whey protein content, which caused a lower rate of drying and higher elasticity of wall matrix. In addition, Laine, et al., (2008) concluded that in the encapsulation of cloudberry extract, a change in DE value did not cause a significant difference in surface morphologies of the capsules.

In the comparison of capsules with MD having DE:13-17 (Fig. 3.12A) and capsules with MD having DE:4-7 (Fig. 3.12B) under ×5000 magnifications, it can be seen that powders with DE:13-17 had more porous structures with more holes within the capsules. The surfaces on the images are the broken microcapsule flakes which were exposed to air. The pores are the residues of entrapped rosemary oil cavitations, resulting from the volatilization of the oil during the exposure. The result is also in accordance with the encapsulation efficiency results (Fig. 3.6) where capsules with WP:MD ratio of 3:1, core to coating ratio of 1:20 and MD having DE:13-17 had higher encapsulation efficiency with less oil left on the surface and more oil trapped inside of the capsules.

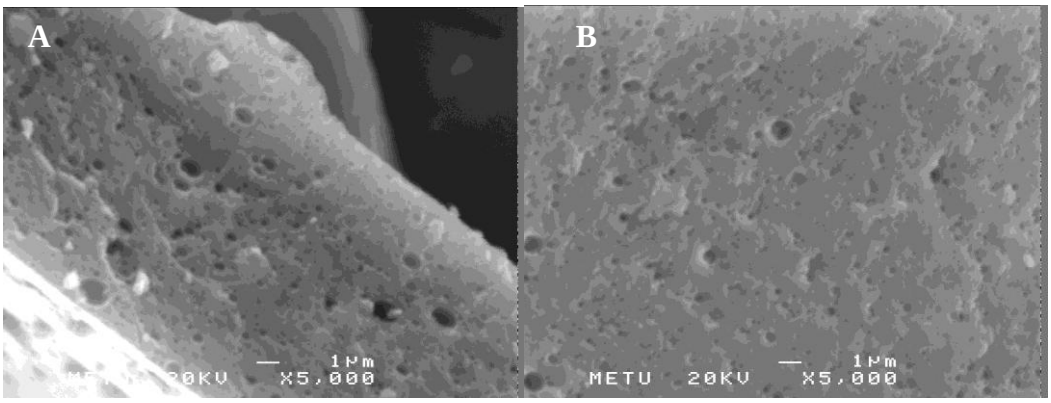

Figure 3.12 Scanning Electron Microscope images (×5000 magnification) of microcapsules prepared with WP:MD ratio of 3:1 and core to coating ratio of 1:20 and maltodextrin having different DE values; (A): DE:13-17, (B): DE:4-7

3.5 GC-MS Analyses during Storage

One of the most important parameters in microencapsulation of volatile oils is the capability of the wall material to retain the volatile compounds during storage. For that, the storage stabilities of the microcapsules having the highest drying and microencapsulation efficiencies, which have WP:MD ratio of 3:1 and core to coating ratio of 1:20, are analyzed and compared with stored non-encapsulated rosemary oil. For the analysis of the extracted oils, GC-MS was used. Also, to see the difference between the storage stabilities of capsules prepared with different DE values, capsules with both DE:13-17 and with DE:4-7 were analyzed. The powders and the fresh rosemary oil were stored under 33.3% relative humidity at 15^0C for 40 days and they were analyzed for their 1,8-cineole concentrations since it is the major component of rosemary oil with the highest concentration. The volume concentrations (μ/μ) were calculated according to the calibration curve prepared by different concentrations of 1,8-cineole (Fig. B.1).

Fig. 3.13 shows the 1,8-cineole concentrations of three samples during 40 days of storage under 33.3% relative humidity. As can be seen from the figure, for all the three samples, the 1,8-cineole concentrations decreased during storage as expected. This decrease of 1,8-cineole was highly due to its volatilization. In the encapsulated oils, the volatilization of 1,8-cineole was lower than non-encapsulated rosemary oil. The reason of this was the barrier effect of the wall materials. Also, the increasing permeability of the wall material allows the oxidation of the encapsulated oil over time which also decreases the 1,8-cineole concentration of encapsulated oil. Especially for the first 10 days of storage, the sharp decrease of 1,8-cineole concentration of non-encapsulated oil shows the barrier effect of the wall materials more clearly. The reported results of Laine et al., (2008) also supported the protective effect of encapsulation of phenolic compounds. According to their research, the non-encapsulated cloudberry phenolics suffered from oxidation and other deteriorative factors of humid environment more than phenolics encapsulated with maltodextrins.

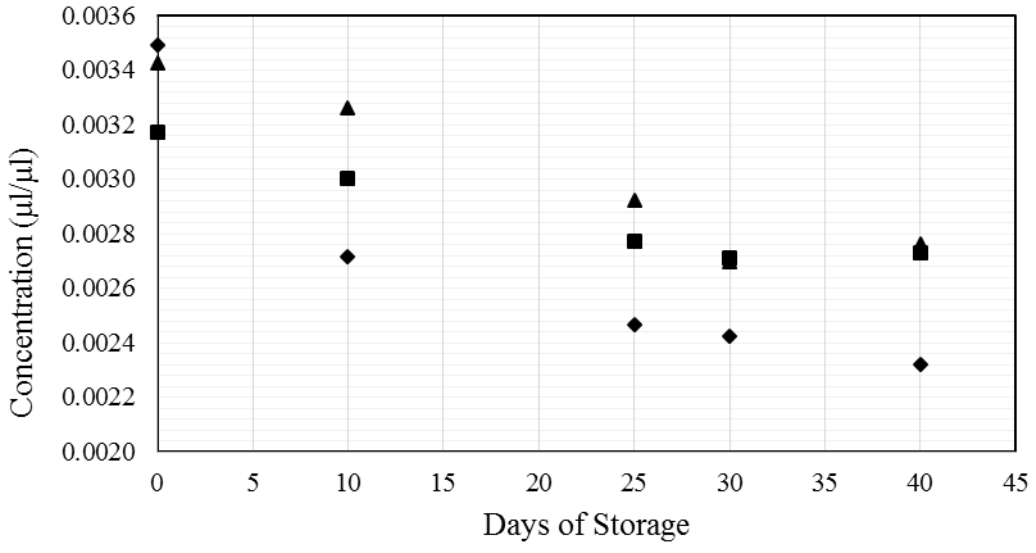

Figure 3.13 1,8-cineole concentration of non-encapsulated oil (♦), capsules prepared with maltodextrin having DE:13-17 (■) and DE:4-7 (▲) for 40 days of storage at 33.3% RH.

The stability performances of microcapsules prepared with maltodextrins having different dextrose equivalent values could also be seen from Fig. 3.13. For the first 30 days of storage, the concentrations of 1,8-cineole for the capsules encapsulated with maltodextrin with DE:4-7 were higher than the concentrations of 1,8-cineole in capsules prepared using MD having DE:13-17. This result indicated that maltodextrin with DE:4-7 achieved the retention of 1,8-cineole better than the maltodextrin with DE:13-17. After 30 days of storage, the effect of different DE values on stability was lost and the retention percentages of maltodextrins became almost equal.

The higher retention performances of lower dextrose equivalent maltodextrins has been investigated by many researchers. According to Ersus & Yurdagel (2007), the worse storage stability of higher dextrose equivalent maltodextrins is due to their lower molecular weight with shorter chains, which are more susceptible to structural deformations than maltodextrins with low DE values. In their study, Anandaraman & Reineccius (1986) also proved that maltodextrin with lower DE value provided better encapsulation properties and longer shelf life for orange peel oil. Moreover, the findings of Wagner & Warthesen (1995), Laine, et al., (2008) and Rodríguez-Hernández, et al., (2005) supported that as the DE value decreased, the storage stability of maltodextrin increased. According to Tonon, et al., (2009), the reason of this difference was the better binding agent properties of lower dextrose equivalent maltodextrins. However, the main reason why lower dextrose equivalent maltodextrins exhibited better storage stability functions was their higher glass transition temperature. As DE value increased, the molecular weight of maltodextrin decreased which also lowered the glass transition temperature of the maltodextrin. Because of this, during storage at high relative humidity environments, high dextrose equivalent maltodextrin had higher hygroscopicity which led to caking and loss of volatile components (Desorby, Netto, & Labuza, 1997).

Fig. 3.14 represents the retention percentages of the compound 1,8-cineole for encapsulated and non-encapsulated oils. It can be also inferred from the figure that encapsulation was very effective in keeping the stability of the core material

during storage. The figure also showed that oil encapsulated with maltodextrin with DE:4-7 was preserved slightly higher than the oil encapsulated with maltodextrin with DE:13-17 because of the reasons explained above.

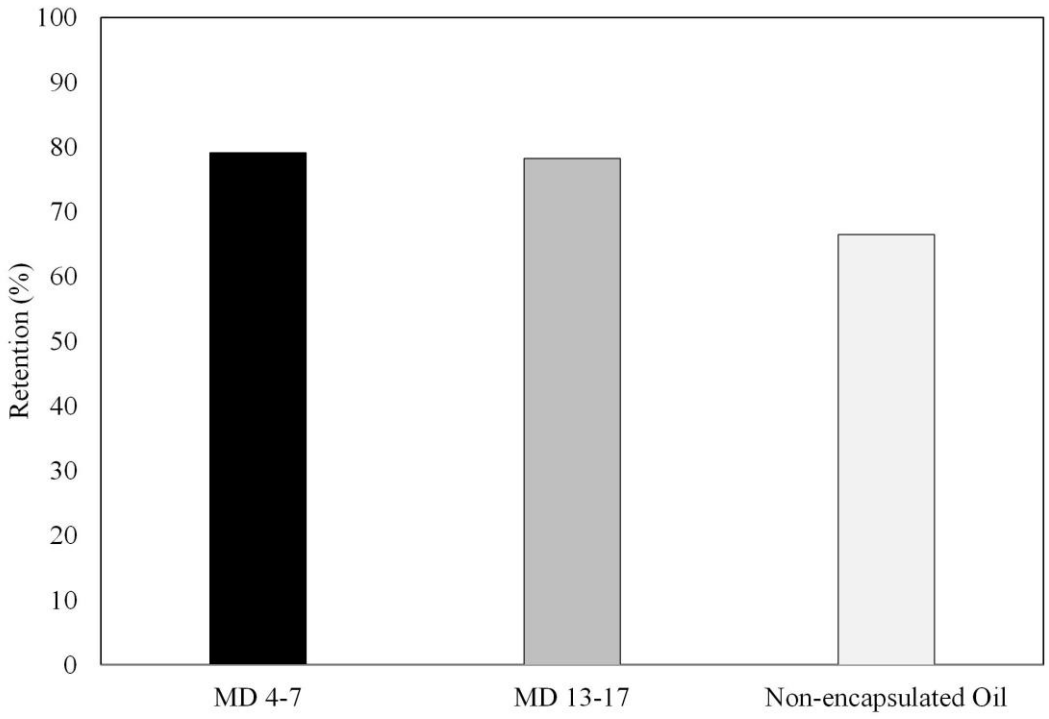

Figure 3.14 Percent retention of 1,8-cineole in capsules prepared with maltodextrin having different DE values; (■): DE:4-7, (■): DE:13-17 and (■): non-encapsulated oil, after storage at 33.3% RH for 40 days.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In order to obtain the best microencapsulation formulation for the encapsulation of rosemary essential oil, drying efficiency and encapsulation efficiency analyses were conducted in the capsules prepared with maltodextrins having two different DE values, different core to coating ratios and WP:MD ratios.

Among the coating formulations, WP:MD ratio of 3:1 provided the highest drying and encapsulation efficiencies for both type of maltodextrin. Increasing core to coating ratio had a decreasing effect on both drying and encapsulation efficiency values. Considering the oil load of the capsules, the highest core to coating ratio giving higher drying and encapsulation efficiencies was found to be 1:20 for both DE values. MD having DE:13-17 was found to yield better drying and encapsulation efficiency values as compared to MD having DE:4-7.

In the particle size analyses of the capsules, DE value did not seem to have any effect on the particle size of the emulsions. However, as core to coating ratio increased, particle size and SSA values increased while span values decreased.

SEM analysis also showed that the DE value did not have any morphological effect on the capsules.

By the GC-MS analyses of stored microcapsules, encapsulation was proven to be very effective on stabilizing the encapsulated rosemary oil during storage. Also, powders prepared with maltodextrin having DE:4-7 was found to have the higher retention values of 1,8-cineole during the first 30 days of storage when compared to powders prepared with maltodextrin having DE:13-17. However, after 30 days of storage, the retention powers of maltodextrins became almost equal. That is why the optimum coating material formulation was chosen to be core to coating

ratio of 1:20 and WP:MD ratio of 3:1 with maltodextrin having DE value of 13-17.

For future study, the effect of different DE values and wall material formulations on the release mechanism of encapsulated rosemary oil could be investigated. Furthermore, to have a better understanding of the hygroscopicity and storage stabilities of different dextrose equivalent maltodextrins, storage stability tests could be conducted at different relative humidity environments. Also, to investigate the physical and chemical changes occurring for the rosemary essential oil during processing, the microcapsules could be put into a food material in the future investigations.

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APPENDIX A

STATISTICAL ANALYSES

Table A.1 Two way ANOVA and Duncan's Multiple Range Test for drying efficiencies of microcapsules prepared using WP:MD ratio of 3:1, core to coating ratios of 1:80, 1:40, 1:20, 1:10 and maltodextrins with different DE values (13-17, 4-7)

X1 Dextrose equivalence (13-17, 4-7)

X2 Core to coating ratio (1:80, 1:40, 1:20, 1:10)

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	4	1 2 3 4

Number of Observations Read 16

Number of Observations Used 16

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2659.646525	664.911631	36.13	<.0001
Error	11	202.447975	18.404361		

Corrected Total	15	2862.094500

R-Square	Coeff Var	Root MSE	Y Mean
0.929266	5.299442	4.290030	80.95250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	319.158225	319.158225	17.34	0.0016
X2	3	2340.488300	780.162767	42.39	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	319.158225	319.158225	17.34	0.0016
X2	3	2340.488300	780.162767	42.39	<.0001

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	11
Error Mean Square	18.40436
Number of Means	2
Critical Range	4.721

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	85.419	8	1

B	76.486	8	2

Duncan's Multiple Range Test for Y

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

Alpha 0.05

Error Degrees of Freedom 11

Error Mean Square 18.40436

Number of Means	2	3	4
Critical Range	6.677	6.984	7.167

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	90.055	4	1
A	90.015	4	2
A	83.165	4	3
B	60.575	4	4

Table A.2 Two way ANOVA and Duncan's Multiple Range Test for drying efficiencies of microcapsules prepared with MD with DE:13-17 at different core to coating ratios and WP:MD ratios

X1 WP:MD ratio (3:1, 1:3)

X2 Core to coating ratio (1:40, 1:20, 1:10)

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

Number of Observations Read 12

Number of Observations Used 12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	7899.968075	2633.322692	167.55	<.0001
Error	8	125.735150	15.716894		
Corrected Total	11	8025.703225			

R-Square	Coeff Var	Root MSE	Y Mean
0.984333	6.585198	3.964454	60.20250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	6521.671875	6521.671875	414.95	<.0001
X2	2	1378.296200	689.148100	43.85	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	6521.671875	6521.671875	414.95	<.0001
X2	2	1378.296200	689.148100	43.85	<.0001

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 8

Error Mean Square 15.71689

Number of Means 2

Critical Range 5.278

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	83.515	6	1
B	36.890	6	2

Duncan's Multiple Range Test for Y

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

Alpha 0.05

Error Degrees of Freedom 8

Error Mean Square 15.71689

Number of Means 2 3

Critical Range 6.464 6.736

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	70.808	4	1
B	64.278	4	2
C	45.523	4	3

Table A.3 Two way ANOVA and Duncan's Multiple Range Test for drying efficiencies of microcapsules prepared using MD with DE:4-7 at different core to coating ratios and WP:MD ratios

X1 WP:MD ratio (3:1, 1:3)

X2 Core to coating ratio (1:40, 1:20, 1:10)

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

Number of Observations Read 12

Number of Observations Used 12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	11145.11523	3715.03841	68.64	<.0001
Error	8	433.01777	54.12722		
Corrected Total	11	11578.13300			

R-Square	Coeff Var	Root MSE	Y Mean
0.962600	17.06988	7.357120	43.10000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	10246.86963	10246.86963	189.31	<.0001
X2	2	898.24560	449.12280	8.30	0.0112

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	10246.86963	10246.86963	189.31	<.0001
X2	2	898.24560	449.12280	8.30	0.0112

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 8

Error Mean Square 54.12722

Number of Means 2

Critical Range 9.795

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	72.322	6	1
B	13.878	6	2

Duncan's Multiple Range Test for Y

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

Alpha 0.05

Error Degrees of Freedom 8

Error Mean Square 54.12722

Number of Means 2 3

Critical Range 12.00 12.50

Means with the same letter are not significantly different.

Duncan Grouping	**Mean**	**N**	**X2**
A	50.280	4	1
A	48.090	4	2
B	30.930	4	3

Table A.4 Two way ANOVA and Duncan's Multiple Range Test for encapsulation efficiencies of microcapsules prepared using MD with DE:13-17 at different core to coating ratios and WP:MD ratios

X1 WP:MD ratio (3:1, 1:3)

X2 Core to coating ratio (1:40, 1:20, 1:10)

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

Number of Observations Read 12

Number of Observations Used 12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	969.075750	323.025250	12.70	0.0021
Error	8	203.424750	25.428094		
Corrected Total	11	1172.500500			

R-Square	Coeff Var	Root MSE	Y Mean
0.826503	5.887825	5.042628	85.64500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	641.2332000	641.2332000	25.22	0.0010
X2	2	327.8425500	163.9212750	6.45	0.0215

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	641.2332000	641.2332000	25.22	0.0010
X2	2	327.8425500	163.9212750	6.45	0.0215

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 8

Error Mean Square 25.42809

Number of Means 2

Critical Range 6.714

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	92.955	6	1
B	78.335	6	2

Duncan's Multiple Range Test for Y

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

Alpha 0.05

Error Degrees of Freedom 8

Error Mean Square 25.42809

Number of Means 2 3

Critical Range 8.222 8.569

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	90.970	4	2
A	87.423	4	1
B	78.543	4	3

Table A.5 Two way ANOVA and Duncan's Multiple Range Test for encapsulation efficiencies of microcapsules prepared using MD with DE:4-7 at different core to coating ratios and WP:MD ratios

X1 WP:MD ratio (3:1, 1:3)

X2 Core to coating ratio (1:40, 1:20, 1:10)

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

Number of Observations Read 12

Number of Observations Used 12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4986.410275	1662.136758	12.58	0.0021
Error	8	1057.399217	132.174902		
Corrected Total	11	6043.809492			

R-Square	Coeff Var	Root MSE	Y Mean
0.825044	17.28811	11.49673	66.50083

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	4829.244408	4829.244408	36.54	0.0003
X2	2	157.165867	78.582933	0.59	0.5745

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	4829.244408	4829.244408	36.54	0.0003
X2	2	157.165867	78.582933	0.59	0.5745

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 8

Error Mean Square 132.1749

Number of Means 2

Critical Range 15.31

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	86.562	6	1
B	46.440	6	2

Duncan's Multiple Range Test for Y

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

Alpha 0.05

Error Degrees of Freedom 8

Error Mean Square 132.1749

Number of Means 2 3

Critical Range 18.75 19.54

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	71.588	4	2
A	64.448	4	3
A	63.468	4	1

Table A.6 Two way ANOVA and Duncan's Multiple Range Test for encapsulation efficiencies of microcapsules prepared using WP:MD ratio of 3:1, core to coating ratios of 1:40, 1:20, 1:10 and maltodextrins having different DE values (13-17, 4-7)

X1 Dextrose equivalence (13-17, 4-7)

X2 Core to coating ratio (1:40, 1:20, 1:10)

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

Number of Observations Read 12

Number of Observations Used 12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	336.6533500	112.2177833	6.98	0.0127
Error	8	128.5552167	16.0694021		
Corrected Total	11	465.2085667			

R-Square	Coeff Var	Root MSE	Y Mean
0.723661	4.466065	4.008666	89.75833

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	122.6241333	122.6241333	7.63	0.0246
X2	2	214.0292167	107.0146083	6.66	0.0198

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	122.6241333	122.6241333	7.63	0.0246
X2	2	214.0292167	107.0146083	6.66	0.0198

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 8

Error Mean Square 16.0694

Number of Means 2

Critical Range 5.337

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	92.955	6	1
B	86.562	6	2

Duncan's Multiple Range Test for Y

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

Alpha 0.05

Error Degrees of Freedom 8

Error Mean Square 16.0694

Number of Means 2 3

Critical Range 6.536 6.812

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	94.838	4	1
B A	89.940	4	2
B	84.498	4	3

Table A.7 Two way ANOVA and Duncan's Multiple Range Test for particle size analyses of emulsions prepared with WP:MD ratio of 3:1, maltodextrins having different DE values and different core to coating ratios.

X1 Dextrose equivalence (4-7, 13-17)

X2 Core to coating ratio (1:40, 1:20, 1:10)

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

Number of Observations Read 6

Number of Observations Used 6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.00421100	0.00140367	106.61	0.0093
Error	2	0.00002633	0.00001317		
Corrected Total	5	0.00423733			

R-Square	Coeff Var	Root MSE	Y Mean
0.993785	1.690337	0.003629	0.214667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	0.00001667	0.00001667	1.27	0.3774
X2	2	0.00419433	0.00209717	159.28	0.0062

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	0.00001667	0.00001667	1.27	0.3774
X2	2	0.00419433	0.00209717	159.28	0.0062

Duncan's Multiple Range Test for Y

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

Alpha 0.05

Error Degrees of Freedom 2

Error Mean Square 0.000013

Number of Means 2

Critical Range .01275

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	0.216333	3	1
A	0.213000	3	2

Duncan's Multiple Range Test for Y

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

Alpha 0.05

Error Degrees of Freedom 2

Error Mean Square 0.000013

Number of Means 2 3

Critical Range .01561 .01492

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	0.250500	2	3
B	0.206000	2	2
C	0.187500	2	1

Table A.8. Two way ANOVA and Duncan's Multiple Range Test for span analyses of emulsions prepared with WP:MD ratio of 3:1, maltodextrins having different DE values and different core to coating ratios.

X1 Dextrose equivalence (4-7, 13-17)

X2 Core to coating ratio (1:40, 1:20, 1:10)

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

Number of Observations Read 6

Number of Observations Used 6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	250.8832967	83.6277656	15.35	0.0618
Error	2	10.8988093	5.4494047		
Corrected Total	5	261.7821060			

R-Square	Coeff Var	Root MSE	Y Mean
0.958367	22.14587	2.334396	10.54100

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	22.8228007	22.8228007	4.19	0.1773
X2	2	228.0604960	114.0302480	20.93	0.0456

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	22.8228007	22.8228007	4.19	0.1773
X2	2	228.0604960	114.0302480	20.93	0.0456

Duncan's Multiple Range Test for Y

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

Alpha 0.05

Error Degrees of Freedom 2

Error Mean Square 5.449405

Number of Means 2

Critical Range 8.201

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	12.491	3	2
A	8.591	3	1

Duncan's Multiple Range Test for Y

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

Alpha 0.05

Error Degrees of Freedom 2

Error Mean Square 5.449405

Number of Means 2 3

Critical Range 10.04 9.597

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	18.791	2	1
B A	8.859	2	2
B	3.973	2	3

Table A.9 Two way ANOVA and Duncan's Multiple Range Test for specific surface area analyses of emulsions prepared with WP:MD ratio of 3:1, maltodextrins having different DE values and different core to coating ratios.

X1 Dextrose equivalence (4-7, 13-17)

X2 Core to coating ratio (1:40, 1:20, 1:10)

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

Number of Observations Read 6

Number of Observations Used 6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	66.62500000	22.20833333	143.28	0.0069
Error	2	0.31000000	0.15500000		
Corrected Total	5	66.93500000			

R-Square	Coeff Var	Root MSE	Y Mean
0.995369	1.388714	0.393700	28.35000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	0.13500000	0.13500000	0.87	0.4492
X2	2	66.49000000	33.24500000	214.48	0.0046

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	0.13500000	0.13500000	0.87	0.4492
X2	2	66.49000000	33.24500000	214.48	0.0046

Duncan's Multiple Range Test for Y

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

Alpha 0.05

Error Degrees of Freedom 2

Error Mean Square 0.155

Number of Means 2

Critical Range 1.383

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	28.5000	3	2
A	28.2000	3	1

Duncan's Multiple Range Test for Y

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

Alpha 0.05

Error Degrees of Freedom 2

Error Mean Square 0.155

Number of Means 2 3

Critical Range 1.694 1.618

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	32.0000	2	1
B	29.1000	2	2
C	23.9500	2	3

Table A.10 One way ANOVA and Duncan's Multiple Range Test for surface oil content (g oil/100 g capsule) of microcapsules prepared with MD having different DE values at different WP:MD and core to coating ratios

X1 Dextrose equivalence (4-7, 13-17)

Class Level Information

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

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	25.40371268	2.30942843	8.62	0.0004
Error	12	3.21333050	0.26777754		
Corrected Total	23	28.61704319			

R-Square	Coeff Var	Root MSE	Y Mean
0.887713	28.80761	0.517472	1.796304

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	11	25.40371268	2.30942843	8.62	0.0004

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	11	25.40371268	2.30942843	8.62	0.0004

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 0.267778

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

Critical Range 1.127 1.180 1.212 1.233 1.248 1.258 1.266 1.271 1.275 1.277 1.279

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	3.8509	2	9
B A	3.1099	2	6
B A	2.6857	2	3
B C	2.3978	2	8
B C	2.3483	2	12
D C	1.4733	2	11
D C	1.3915	2	5
D C	1.3594	2	10
D C	1.2230	2	4
D	0.6443	2	2
D	0.5852	2	7
D	0.4864	2	1

APPENDIX B

CALIBRATION CURVE

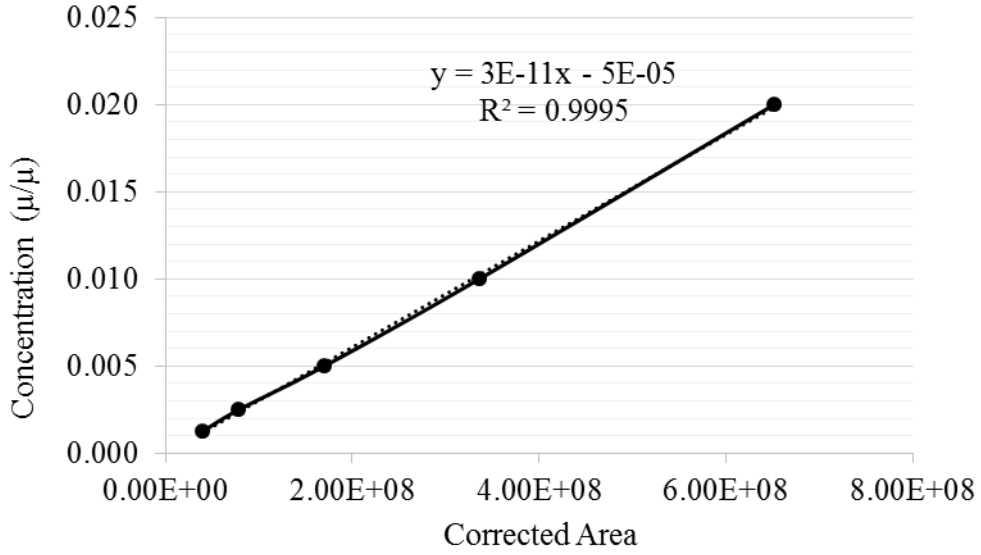

Figure B.1 Calibration curve for 1,8-cineole concentrations of stored microcapsules and non-encapsulated oil.