OCTOBER 2020 Ph.D. in Food Engineering AYSEL ELİK

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ENCAPSULATION OF FLAXSEED OIL ENRICHED IN CAROTENOID BY MICROWAVE TECHNIQUE USING INNOVATIVE WALL MATERIALS

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BY

AYSEL ELİK

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

Aysel ELİK

ABSTRACT

ENCAPSULATION OF FLAXSEED OIL ENRICHED IN CAROTENOID BY MICROWAVE TECHNIQUE USING INNOVATIVE WALL MATERIALS

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In this study, flaxseed oil enriched in carotenoid by microwave technique was encapsulated using innovative wall materials. In the first part of the study, the effect of microwave technology and the use of flaxseed oil as the solvent on the yield of carotenoid extraction from carrot juice processing waste were investigated. Response surface methodology was employed to optimize the effect of microwave power (50-200 W), extraction time (1-12 min) and oil to solid ratio (5:1-20:1 g/g) on percentage recovery of carotenoid. Optimum conditions obtained for microwave-assisted carotenoid extraction were 165 W, 9.39 min and 8.06:1 g/g of oil to solid ratio which gave carotenoid recovery of 77.48%. Selected physical and chemical properties of flaxseed oil and carotenoid enriched flaxseed oil were investigated in terms of fatty acid composition, acid value, peroxide value, p-anisidine value, colour parameters (Hunterlab L*, a* and b* values), β-carotene content, total phenolic content and antioxidant activity (DPPH). Encapsulation of the enriched flaxseed oil was performed by spray freeze drying by using different emulsion formulations. Emulsions which contain various concentrations of carotenoid enriched flaxseed oil (6, 9, 12 and 15% w/w) and ratios of maltodextrin/(pectin+wax) proportions (3:1, 6:1, 9:1 and 12:1 g/g) were encapsulated by spray freeze drying to determine the emulsion formulation with the highest encapsulation efficiency. Spray freeze drying and spray drying techniques were compared in terms of the encapsulation efficiency, moisture content, particle size, flowing properties, morphological properties and oxidative stability.

Key Words: Carotenoid, Microwave Assisted Extraction, Pectin, Wax, Spray Freeze Drying

ÖZET

MİKRODALGA TEKNİĞİYLE KAROTENOİD AÇISINDAN ZENGİNLEŞTİRİLMİŞ KETEN TOHUMU YAĞININ YENİLİKÇİ DUVAR MATERYALLERİ KULLANILARAK ENKAPSÜLASYONU

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Bu çalışmada, mikrodalga tekniğiyle karotenoid açısından zenginleştirilmiş keten tohumu yağı yenilikçi duvar materyalleri kullanılarak enkapsüle edilmiştir. Çalışmanın ilk bölümünde, mikrodalga teknolojisi ve çözücü olarak keten tohumu yağı kullanımının, havuç suyu proses atığından karotenoid ekstraksiyon verimi üzerindeki etkisi incelenmiştir. Mikrodalga gücü (50-200 W), ekstraksiyon süresi (1-12 dakika) ve yağ/katı madde oranının (5:1-20:1 g/g) yüzde karotenoid ekstraksiyon verimi üzerindeki etkisini optimize etmek için yanıt yüzey metodolojisi kullanılmıştır. Mikrodalga destekli karotenoid ekstraksiyonu için elde edilen optimum koşullar 165 W, 9,39 dakika ve 8,06:1 g/g yağ/katı madde oranı olup, %77,48 oranında karotenoid ekstraksiyon verimi sağlanmıştır. Keten tohumu yağı ve karotenoid ile zenginleştirilmiş keten tohumu yağı, yağ asidi kompozisyonu, serbest asitlik değeri, peroksit değeri, p-anisidin değeri, renk parametreleri (Hunterlab L*, a* ve b* değerleri), β-karoten içeriği, toplam fenolik içeriği ve antioksidan aktivitesi (DPPH) açısından karşılaştırılmıştır. Zenginleştirilmiş keten tohumu yağının enkapsülasyonu, farklı emülsiyon formülasyonları kullanılarak püskürtmeli dondurarak kurutma ile gerçekleştirilmiştir. Çeşitli konsantrasyonlarda zenginleştirilmiş keten tohumu yağı (%6, %9, %12 ve %15 m/m) ve maltodekstrin/(pektin+vaks) oranları (3:1, 6:1, 9:1 ve 12:1 g/g) içeren emülsiyonlar en yüksek enkapsülasyon etkinliğine sahip emülsiyon formülasyonunu belirlemek için püskürtmeli dondurarak kurutma ile enkapsüle edilmiştir. Püskürtmeli dondurarak kurutma ve püskürtmeli kurutma teknikleri enkapsülasyon etkinliği, nem içeriği, partikül boyutu, akış özelliği, morfolojik özellikler ve oksidatif stabilite açısından karşılaştırılmıştır.

Anahtar Kelimeler: Karotenoid, Mikrodalga destekli ekstraksiyon, Pektin, Vaks, Püskürtmeli dondurarak kurutma

"dedicated to my beloved mom"

AYŞETE ELİK

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CCE	Conventional Carotenoid Extraction
CCRD	Central Composite Rotatable Design
CE	Amount of Carotenoid Extracted
CEFO	Carotenoid-Enriched Flaxseed Oil
CI	Carr Index
CSO	Corrected Surface Oil Content
CPW	Carrot Juice Processing Waste
CW	Total Carotenoid Content of CPW
DHA	Docosahexaenoic Acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FO	Flaxseed Oil
HO	Hausner Ratio
HPLC	High Performance Liquid Chromatography
KOH	Potassium Hydroxide
LMP	Low Methoxylated Pectin
MACE	Microwave Assisted Carotenoid Extraction
MD	Maltodextrin
NaCI	Sodium Chloride
Na_2CO_3	Sodium Carbonate
ND	No Data
PUFA	Polyunsaturated Fatty Acid
PV	Peroxide Value
RSM	Response Surface Methodology
SD	Spray Drying
SEM	Scanning Electron Microscopy

SFD	Spray Freeze Drying
SFW	Sunflower Wax
TBARS	Thiobarbituric Acid Reactive Substances
TLC-FID	Thin Layer Chromatography and Flame Ionization Detector
TO	Total Oil Content
TPC	Total Phenolic Content

CHAPTER 1

INTRODUCTION

Flaxseed oil (FO) is one of the richest sources of omega-3 fatty acids. It has a chemical composition constituting 9% of saturated fatty acids, 18% of monounsaturated fatty acids and 73% of polyunsaturated fatty acids (Goyal et al., 2014). Oils with high polyunsaturated fatty acids, such as FO, have many beneficial effects such as preventing many diseases (Vijaimohan et al., 2006). However, FO is easily oxidized because of its high PUFAs content and this limits its utilization in the food industry (Sharif et al., 2017). Therefore, oils rich in PUFAs are generally encapsulated in a polymeric matrix to increase oxidative stability thereby providing longer shelf life. Moreover, the development of novel functional foods which are enriched or fortified with lipophilic bioactives has gained popularity in order to improve well-being and health of consumers. Therefore, enrichment of omega oils with antioxidants such as carotenoids is preferable for promoting the oxidative stability of the encapsulated oil during processing and storage stages (Sharif et al., 2017). In the present study, FO was enriched with carotenoids using by microwave technique before encapsulation process.

Carrot (*Daucus carota* L) is one of the most important root fruits grown worldwide and is a good source of carotenoid (Sharma et al., 2012). However, in the production of carrot juice, the pulp is wasted corresponding to 30–40% of the raw material. The process waste of carrot is rich in bioactive substances such as carotenoids, especially in β-carotene (Oreopoulou and Russ, 2007). Depending on the process conditions, the total carotene content of this waste can reach up to 2 g per kg dry matter (Dhillon and Kaur, 2016).

Recovery of carotenoids in wastes is generally carried out using organic solvents such as hexane and acetone which are harmful to the environment and human health. However, carotenoids are oil-soluble pigments (Sachindra and Mahendrakar, 2005) and thus the use of oil as a solvent can be a promising alternative method to organic solvents. The use of oil as alternative solvent has the advantages such as reducing the

use of environmentally harmful solvents and eliminating the extra cost of evaporation. Additionally, barrier role of the oil against oxygen leads to retardation of the oxidation time and degradation rate for the extract containing carotenoids (Parjikolaei et al., 2015).

There are some studies in the literature, dealing with the carotenoid extraction using oil as a solvent. However, most of these studies are based on the conventional solvent extraction process with a long extraction time (1 to 48 hours) (Sachindra and Mahendrakar, 2005; Benakmoum et al., 2008; Handayani et al., 2008; Kang and Sim, 2008; Kessy et al., 2011; Parjikolaei et al., 2015). In recent years, ultrasound technology and vegetable oils have been used in a few studies in order to extract carotenoids. They have been reported to provide better extraction yield (up to about 94 %) and shorter time (max. 30 min) when compared to conventional processes (Y. Li et al., 2013; Goula et al., 2017).

Microwave extraction can be regarded as one of the most appropriate method for β-carotene extraction from carrots (Kyriakopoulou et al., 2015) due to less time consumption and higher extraction efficiency. When the carotenoid extraction studies used vegetable oils as a solvent are examined, it turns out that the main drawback is long extraction time. The microwave shortens the duration of the extraction by causing rapid disruption of the cell walls and increases the yield of the extraction (Milutinović et al., 2015). However, there is no exhaustive study available on the carotenoid extraction from carrot juice processing waste (CPW) using oil as a solvent under the microwave conditions.

Spray drying (SD), the oldest and a well-established process, is widely used for the encapsulation of flavors and oils (Jafari et al., 2008; Can Karaca et al., 2013; Carneiro et al., 2013; Pellicer et al., 2019). It has many advantages such as low processing cost, compatible with many wall materials and applicable to continuous operation (Anandharamakrishnan et al., 2007; Anandharamakrishnan et al., 2008; Sosnik and Seremeta, 2015). However, in SD, core materials are inevitably exposed to high temperature and this might lead to significant oxidation of PUFAs which are sensitivity to heat. Hence, the alternative and new approaches with low temperature processes like spray freeze drying (SFD) need to be investigated in order to protect omega oils against oxidation during process.

The potential of SFD is still being explored in the pharmaceutical and the food industries. However, SFD is a promising process especially for encapsulation of heat-sensitive substances since the process is executed at low temperature (Ishwarya et al., 2015). Some other advantages of SFD could be counted as the production of porous powders with higher dispersibility and flowability compared to SD technique (Liang et al., 2018). Although it has a great potential for the encapsulation of oxidation-sensitive oils such as FO, there is only one reported study which is used SFD for the encapsulation of DHA oil (Karthik and Anandharamakrishnan, 2013).

The type of wall material is a critical factor for the encapsulation process (Cao et al., 2020). Synthetic or natural polymers are generally used as wall material (usually polysaccharides and proteins) in the encapsulation of oils. The selection of suitable wall materials is very important as they affect the encapsulation efficiency and the characteristics of the powder.

Pectin is a carbohydrate mixture found in the wall cell of many plants and can form stable emulsions at low concentrations (Ngouemazong et al., 2015). The emulsion activity of pectin is due to its ability to reduce the interfacial tension between oil and water phases by forming a protective layer around the oil droplets to prevent them from aggregation (Leroux et al., 2003). However, earlier reports also suggested lower effect of emulsion stabilization of pectin because of the relatively thin hydrated layer unable to provide sufficient steric effects (Nakauma et al., 2008; Maravic et al., 2019). Therefore, addition of maltodextrin to emulsions systems along with emulsifying agent results in more stable emulsion (Klinkesorn et al., 2004) as maltodextrin can exhibit certain specific stabilizing properties (Maravic et al., 2019).

Macromolecules of pectin are inefficient as a barrier against water vapor due to their hydrophilic capacity (Baumler et al., 2013). Waxes, esters of long chain alcohols and fatty acids, are widely used as an edible coating material to improve the water vapor and oxygen barrier properties of food products (Bourlieu et al., 2009). Moreover, the presence of wax crystals might contribute further to the stabilization of the emulsion formed if crystals are in the continuous phase, coming into contact with the interface and adsorbing at the surface of the droplet (C. F. Li et al., 2009). Thus, the combination of pectin with other polysaccharides (e.g. maltodextrin) and lipids (e.g. wax) can be effective in increased emulsion stability and barrier

characteristics (Nakauma et al., 2008; Chalapud et al., 2018). Although there are many studies on the encapsulation of FO, no study has been reported on the use of the maltodextrin, pectin and wax combinations as a wall material.

The main purposes of this thesis are follows;

- to investigate the effect of microwave technology on carotenoid recovery from carrot juice processing waste (CPW) by using FO as a solvent and to optimize the extraction conditions (microwave power, extraction time and oil to solid ratio) to achieve optimum recovery of carotenoids.
- to carry out the comparative studies between microwave-assisted carotenoids extraction (MACE) and conventional carotenoid extraction (CCE) using FO as a solvent in terms of recovery of carotenoids and extraction time.
- to compare fatty acid composition, colour parameters (L*, a*, b*), acid value, peroxide value, p-anisidine value, total phenolic content, β-carotene content and antioxidant activity (inhibition % of DPPH) of carotenoid-enriched flaxseed oil (CEFO) obtained under optimum conditions to those of FO.
- to determine the effect of various compositions of maltodextrin, pectin and wax as wall materials on the encapsulation efficiency of CEFO by SFD.
- to compare two encapsulation techniques, SFD and SD, for the best emulsion (with the highest encapsulation efficiency defined in SFD technique) for their encapsulation efficiency, moisture content, particle size, flowing properties and morphology of the powder products.
- to investigate the effect of carotenoid presence in FO (CEFO) on the oxidative stability during processing and storage for the encapsulated products by SFD and SD techniques.

CHAPTER 2

LITERATURE REVIEW

2.1 Carrot Processing Waste

Carrot (*Daucus carota* L) is one of the most important root fruits grown worldwide and is an important source of carotenoids (Sharma et al., 2012). In 2018, world production of carrot amounted to approximately 40 million tons. China is the leading carrot producing country in the world. Other major producers were Uzbekistan, United States and Russia (FAOSTAT, 2018). The consumption of carrot and its products have recently increased due to their biological activities such as antioxidant, anticarcinogenic activity (Sharma et al., 2012).

Carrot juice is an important processed product of carrot and among one of the most popular non-alcoholic beverages in various countries (Schieber et al., 2001). In the production of carrot juice, a pulp is wasted corresponding to 30-40% of the raw material (Figure 2.1). The resultant pulp is disposed as manure or feed (Sharma et al., 2012). However, the process waste of carrot is rich in bioactive substances such as carotenoids, especially in β-carotene (Oreopoulou and Russ, 2007). Depending on the processing conditions, the total carotene content of this waste can reach up to 2 g per kg dry matter (Dhillon and Kaur, 2016). In addition, these wastes not only create further sources for pollution generation, but also lead to loss of valuable biomass and nutrients (Sharma et al., 2012). Hence, new technologies are needed to explore for decreasing the global volume of food waste. The valorization of food waste through value-added products can be an ideal and practical solution for decreasing the global volume of food waste (Tsang et al., 2019).

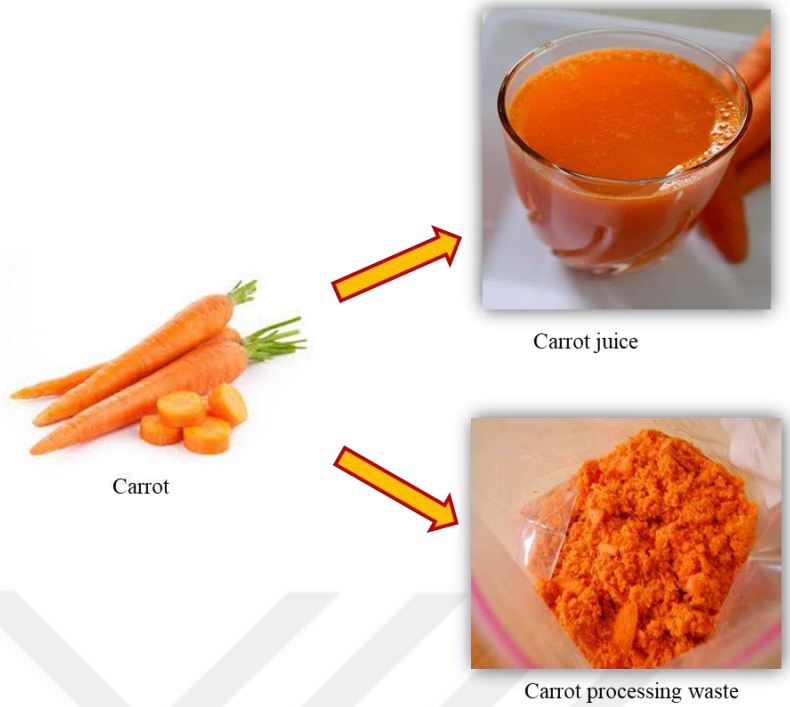


Figure 2.1 Carrot juice and carrot processing waste after production of juice

2.2 Flaxseed Oil

Flaxseed or linseed is one of the oldest cultivated crops in the world (Goyal et al., 2014). It is the seed from the flax plant (*Linum usitatissimum*) which is a member of the *Linaceae* family (Herchi et al., 2012). Canada is the largest producer of flaxseed in the world with approximately 0.8 tons in 2014-2018, followed by Kazakhstan and Russia (FAOSTAT, 2018). Figure 2.2 shows total average world production of flaxseed in 2014-2018 (FAOSTAT, 2018).

Flaxseed has been historically used for a variety of diseases such as abdominal pain, upper respiratory, skin inflammation and constipation (Basch et al., 2007). It was first introduced in United States by colonists, primarily to produce fiber for clothing. In the last two decades, flaxseed has been the focus of increased interest in the field of diet and disease research due to the potential health benefits associated with some of its biologically active components (Goyal et al., 2014).

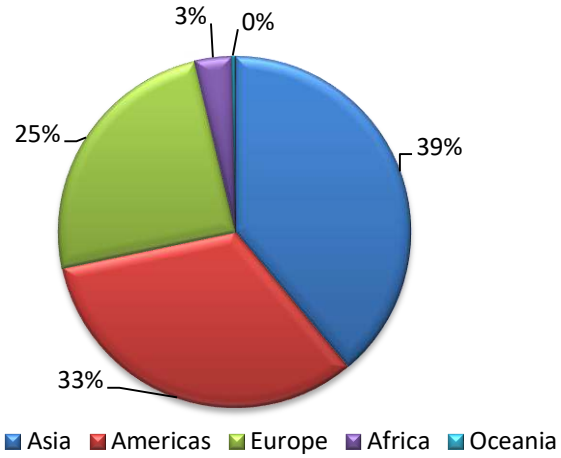


Figure 2.2 Average production of linseed/flaxseed in the world in 2014–2018 (FAOSTAT, 2018)

Flaxseed is rich in oil, fiber, protein vitamins and minerals. Flaxseed contains averagely 30 to 41% oil, 20 to 35% total dietary fiber, 20 to 30% protein, 4 to 8% moisture and 3 to 4% ash. Vitamins A, B, D and E, amino acids and minerals are also found in the oil from seed (Goyal et al., 2014; Bekhit et al., 2018).

FO extracted from seeds from the plant is a rich source of polyunsaturated fatty acids (PUFAs), which account for 73% of fatty acids in the oil. Oils with the high PUFAs such as FO play an important role in the prevention of many diseases (Oomah, 2001). Table 2.1 show the major fatty acid profile in FO. Moreover, it contains at a high amount of natural antioxidants like β-carotene and tocopherols (Goyal et al., 2014).

Table 2.1 Major fatty acids profile in flaxseed oil (Goyal et al., 2014)

Fatty acid	Percentage % (range)
Palmitic acid (C16:0)	4.9-8.00
Stearic acid (C18:0)	2.24-4.59
Oleic acid (C18:1)	13.44-19.39
Linoleic acid (C18:2)	12.25-17.44
Linolenic acid (C18:3)	39.90-60.42

2.3 Carotenoids

Carotenoids are the natural pigments that are synthesized by living organism like plants, algae, several fungi and bacteria. These pigments produce red, yellow, and orange colors in plants, fruits and vegetables. Carotenoids may be splitted into two groups, namely carotenes (e.g. β-carotene and lycopene) and xanthophylls (e.g. lutein and zeaxanthin). Xanthophylls (phylloxanthins), oxygenated derivatives carotenes, are yellow pigments that occur widely in nature. They differ from carotenes as they possess one or more attached groups which contain oxygen. In addition, xanthophylls are more polar than carotenes. They are synthesized within the plastids and are found in the leaves of most plants. On the other hand, carotenes are orange photosynthetic pigments which are responsible for the orange colour of many fruits and vegetables such as of the carrot (Amorim-Carrilho et al., 2014). Some of the most important carotenoid compounds are shown in Figure 2.3.

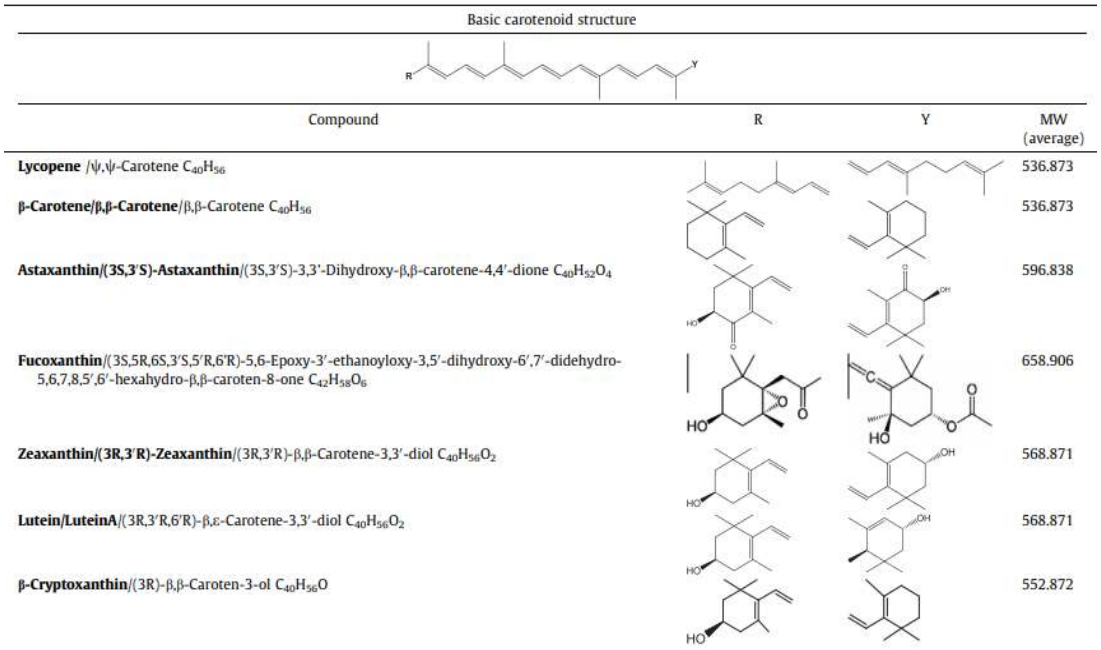


Figure 2.3 Chemical formulas, molecular weights (MW) and structures of some of the main carotenoid compounds (Amorim-Carrilho et al., 2014)

Almost all carotenoids act as antioxidants in the human body (Amorim-Carrilho et al., 2014). They are used as a source for vitamin A (Stahl and Sies, 2003). They have the beneficial health-promoting effects such as prevention of heart cancer, vascular disease (Agarwal et al., 2012), degenerative diseases and other chronic diseases (e.g. cataracts and age-related macular degeneration) (Obulesu et al., 2011; Wegner and

Khoramnia, 2011; Sabour-Pickett et al., 2012). The preventive effects of carotenoids for many diseases mentioned above are attributed to their antioxidant activity, protecting tissues and cells from oxidative damage (Stahl and Sies, 2003).

2.3.1 Extraction Methods of Carotenoid

2.3.1.1 Conventional Extraction Method

Different conventional extraction methods are used for the extraction of carotenoids from various samples. Most of these methods are based on the application of heat and/or mixing and the extraction power of solvent used (Azmir et al., 2013). Conventional techniques are the most commonly used extraction processes although they have some disadvantages. Table 2.2 describes the main advantages and drawbacks of various extraction methods.

Among the conventional methods, the most commonly used carotenoid extraction methods are maceration and soxhlet extraction.

Maceration is a simple extraction method that consists of three steps. In the first step, sample is grinded into small particles to increase the surface area and thereby ensuring that the sample is efficiently mixed with solvent. In the following step, sample is soaked in an appropriate solvent under room temperature with occasional or continuous agitation. Finally, solid residue is separated from liquid fraction by clarification, filtration or decantation. The extraction yield is increased when the agitation is applied in the maceration process. This is attributed to increase the diffusion and to remove the concentrated solution from the sample surface which increases the contact of new solvent with the sample (Aires, 2017). This method is very simple to use, but it has some disadvantages, such as requiring a large amount of solvent and being time consuming (Table 2.2).

Soxhlet extraction method has been widely used for the extraction of carotenoids and provides the highest recovery of carotenoids. Therefore, it is commonly used as a reference method for evaluating the performance of other extraction methods. However, it has some disadvantages such as being time consuming and requiring large amounts of solvents, thus increasing the cost of extraction. Moreover, long extraction time and the high temperature increase the possibility of thermal degradation of carotenoids (Saini and Keum, 2018).

In the conventional extraction methods, the solvent selection is the most important issue for an efficient extraction. The polarity of the targeted component is the main factor in the selection of solvent. Moreover, chain length of the existing carotenoids, moisture content, the sample components and its matrix should be considered when selecting solvent. Organic solvents, such as hexane, acetone, isopropanol, chloroform, diethyl ether, methanol and methylene chloride are commonly used for the carotenoid extraction. However, some green solvent such as vegetable oils, which have been discussed in subsequent sections, are explored to use for the carotenoid extraction due to health, safety and environmental issues (Saini and Keum, 2018).

2.3.1.2 Non-Conventional Extraction Methods

The major disadvantages of the conventional extraction techniques are long processing time, the possibility of thermal decomposition of heat-sensitive compounds, cost, low extraction selectivity, requirement of large amount of expensive solvents for extraction and the need to evaporate large amounts of solvent. New and promising extraction methods have been recently developed to tackle these issues. Ultrasound-assisted extraction, enzyme-assisted extraction, microwave-assisted extraction, pressurized liquid extraction and supercritical fluid extraction are some of the most promising non-conventional extraction techniques.

The ultrasound-assisted extraction method utilizes high frequency sound waves to destroy the walls of the plant that enhances the mass-transfer of extractants. The efficiency of ultrasound-assisted extraction depends on temperature, intensity, ultrasonic power and solvent to sample ratio. Ultrasound-assisted extraction method is relatively simple, easy, fast, low in cost, has high efficiency and consumes low organic solvent (Abduljabar et al., 2019). The ultrasound-assisted extraction method has been successfully used to extract carotenoids from various samples by using both organic solvents (Dey and Rathod, 2013; Luengo et al., 2014) and vegetable oils (Ordóñez-Santos et al., 2015; Goula et al., 2017) as the extraction solvents. Despite its advantages, this method suffers from many problems such as decline of power with time, the lack of uniformity in the distribution of ultrasound energy and filtration requirement after the extraction (Abduljabar et al., 2019).

Supercritical fluid extraction is one of the alternative methods to the conventional extraction technique for the extraction of carotenoids. Unlike normal solvent

extraction, in the supercritical fluid extraction, solvents are used in their supercritical state. Supercritical CO_2 is the most commonly used supercritical fluid. The advantages in extraction with CO_2 include its chemical inertness, lack of toxicity, ready availability, low cost and suitable method for thermolabile components. In recent years, this technology has been widely studied for the recovery of pigments from marine microalgae (Macias-Sanchez et al., 2005).

Pressurized liquid extraction also known as accelerated fluid extraction, pressurized fluid extraction, high pressure solvent extraction and enhanced solvent extraction. It is based on applying high pressure, penetration of the extracting solvent, facilitating improved cell permeability and intermolecular physical interactions, and thus enhances extraction yield of carotenoids (Saini and Keum, 2018). Furthermore, the combination of high temperatures and pressure ensures faster extraction. Pressurized liquid extraction has been used successfully for the carotenoid extraction from various samples such as lyophilized apricot (*Prunus armeniaca*), peach (*Prunus persica* L.), Tunisian Kaki (*Diospyros kaki*) (Zaghdoudi et al., 2015) and microalga (*Neochloris oleoabundans*) (Castro-Puyana et al., 2013).

Enzyme-assisted extraction method uses hydrolytic enzymes for the degradation of cell walls and thus exposes intracellular materials for increased recovery of bioactive compounds. It is one of the methods that have become popular in last years for the carotenoid extraction (Saini and Keum, 2018). Cellulase and pectinase enzymes are generally employed to degrade cell wall in a pre-treatment stage prior to solvent extraction, thus they enhance the extraction of natural food pigments such as carotenoids (Saini and Keum, 2018; Lombardelli et al., 2020). Strati et al. (2015) repoted that the recovery of carotenoids from tomato paste increased considerably (up to 10-fold) when the sample was treated with cellulase and pectinase before the solvent extraction. Nath et al. (2016) studied the effect of three carbohydrases enzymes (viscozyme L, cellulose and pectinase) on the recovery of carotenoids from red capsicum (*Capsicum annuum*). The viscozyme L with multi-enzyme preparations was suggested a very effective and promising enzyme for providing the high recovery of carotenoids.

Table 2.2 Advantages and drawbacks of various carotenoid extraction methods (Saini and Keum, 2018)

Extraction Method	Advantages	Drawbacks
Maceration	• Providing high recovery without requiring sophisticated instruments	• Cost of production due to requirement of large amounts of toxic solvents • Time-consuming
Soxhlet extraction	• Simple and no sophisticated instruments required • Providing high recovery of carotenoids	• Can cause thermal degradation • Requiring to evaporate the large amount of solvent • Requiring large amounts of solvents, which increases the cost of extraction • Time-consuming
Ultrasound-assisted extraction	• Providing efficient, rapid and non-thermal extraction • Low energy usage	• Aging of the ultrasonic probe surface can change the extraction efficiency • Filtration requirement after the extraction
Pressurized liquid extraction	• Rapid and requires smaller volume of organic solvent	• Difficult to apply to large volumes because of clogging caused by pectins and sugars of plant matrices
Supercritical fluid extraction	• Utilization of non-toxic, non-flammable and recyclable solvent • Applicable to continuous extraction system • Providing carotenoids with high purity • Useful for extraction of heat-sensitive compounds	• Not suitable for samples with high amounts of water • High cost of instrumentation • Low yield of polar carotenoids
Enzyme-assisted extraction	• Efficient and rapid process • Requiring smaller volume of organic solvent	• High cost of the enzymes
Microwave-assisted extraction	• Simple, fast and economical method	• Can cause thermal degradation

2.3.1.2.1 Microwave Assisted Extraction

Microwave assisted extraction (MAE) is an extraction method which utilizes microwave radiation to allow the partition of analytes from the sample into the extraction solvent (Dai and Mumper, 2010). Microwave heating in solution can occur due to the induction of molecular movements which lead to rupture the cell wall and to release the phytochemicals inside the cell. Microwave energy absorbed by materials depends on their dielectric properties (Regier et al., 2017). Polar solvents such as water have a large electric dipole moment than non-polar solvents such as oils, and thus they can absorb much higher energy then non-polar solvents.

In MAE, there are two extraction systems, namely open vessels (at atmospheric pressure) and closed vessels (under controlled pressure and temperature) (Camel, 2001; Venkatesh and Raghavan, 2004). While the closed system is employed at elevated temperature and pressure, the extraction is carried out in the open system at atmospheric pressure that the maximum temperature depends the boiling point of the solvent or solvent combination used in the extraction (Venkatesh and Raghavan, 2004; Stolker and Danaher, 2011). Furthermore, the efficiency of MAE strongly relies on some parameter such as microwave power, extraction time, solvent to feed ratio and sample characteristic (Chan et al., 2011).

MAE is a simple, fast and efficient technique for the carotenoid extraction. It requires a low amount of solvents and very short extraction time (Saini and Keum, 2018). There are a lot of studies on MAE of carotenoids from various sources by using organic solvents. Ho et al. (2015) used MAE for the lycopene extraction from tomato peels using ethyl acetate as the extraction solvent. They suggested that ethyl acetate is a better solvent for MAE due to its higher recovery of carotenoid compared to hexane.

The key factor affecting extraction yield of carotenoid is the structure of the material. It was found that applying certain pre-treatments prior to MAE is beneficial in increasing the extraction yield of carotenoids (Saini and Keum, 2018). Bhudsawan Hiranvarachat et al. (2013) explored the effect of the pre-treatment on the sample structure and the recovery of carotenoids from carrot before MAE. They obtained that the antioxidant activity and the carotenoid recovery from carrots blanched in citric acid and water were significantly higher than that of untreated carrots. This was

explained by damaging and softening the sample structure, possibly by heat mediated solubilization of pectin. Esquivel-Hernández et al. (2016) compared the efficiency of MAE and supercritical carbon dioxide fluid extraction to extract the functional lipophilic compounds such as carotenoids and tocopherols from *Arthrospira platensis* biomass. Results showed that MAE was more effective than supercritical carbon dioxide fluid extraction in terms of the extraction yield of carotenoids.

2.3.1.3 Carotenoid Extraction Using Vegetable Oils

Carotenoid extraction processes are generally carried out by using the solvents which are obtained from non-renewable resources. These solvents are highly volatile, flammable and often toxic, leading to the greenhouse effect and environmental pollution. Hence, green solvents which are environmentally friendly become popular because they are renewable resources produced from petrochemical products that are biodegradable and non-toxic or from biomass feedstock such as fruits, starch, wood and vegetable oils (Saini and Keum, 2018).

Carotenoids are oil-soluble pigments (Sachindra and Mahendrakar, 2005) and the use of vegetable oil as solvent can be a promising alternative method to organic solvents. The use of oil as alternative solvent has the advantages such as reducing the use of environmentally harmful solvents and eliminating the extra cost of evaporation. Additionally, barrier role of the oil against oxygen leads to retardation of the oxidation time and degradation rate for the extract containing carotenoids (Parjikolaei et al., 2015).

There are some studies in the literature, dealing with the carotenoid extraction using oil as a solvent (Table 2.3). Parjikolaei et al. (2015) used methyl ester of sunflower oil and sunflower oil which are green extraction solvents for extraction of astaxanthin from shrimp processing waste (*Pandalus borealis*). The authors reported 60% and 80% recovery of astaxanthin using sunflower oil and methyl ester of sunflower oil, respectively. They suggested that the vegetable oil product which is saturated with carotenoid could be utilized directly in the diet.

Benakmoum et al. (2008) evaluated three green solvents, extra virgin olive oil, refined olive oil and refined sunflower oil, for the extraction of carotenoids from

tomato purée. They suggested that the extraction of carotenoids into low quality edible oils is a good approach for the valorization of tomato by-products.

However, most of these studies are based on the conventional solvent extraction process with a long extraction time (1 to 48 hours) (Sachindra and Mahendrakar, 2005; Benakmoum et al., 2008; Handayani et al., 2008; Kang and Sim, 2008; Kessy et al., 2011; Parjikolaei et al., 2015).

In recent years, ultrasound technology and vegetable oils have been used in a few studies in order to extract carotenoids. (Y. Li et al., 2013) developed a process for ultrasound-assisted extraction of carotenoids from fresh carrots using sunflower oil as solvents. They compared ultrasound-assisted extraction technique using vegetable oil as solvent and conventional solvent extraction technique using hexane as solvent. They obtained that the ultrasound-assisted extraction using sunflower as solvent has obtained its highest β-carotene yield (334.75 mg/l) in 20 min only, while conventional solvent extraction technique using hexane as solvent obtained a similar yield (321.35 mg/l) in 60 min.

Goula et al. (2017) also used ultrasound-assisted extraction process for the extraction of carotenoids from pomegranate peels using soy oil and sunflower oil as extraction solvents. Approximately 85.7 and 93.8% of the total carotenoids present in the waste material were extracted by ultrasound-assisted extraction using soy oil and sunflower oil, respectively. They suggested that the diffusivity of carotenoids can be enhanced by the ultrasound. Thus the major limitation in the usage of vegetable oil as an extraction solvent can be eliminated using ultrasound technology.

Microwave extraction can be regarded as one of the most appropriate method for β-carotene extraction from carrots (Kyriakopoulou et al., 2015) (due to less time consumption and higher extraction efficiency. When the carotenoid extraction studies used vegetable oils as a solvent are examined, it is considered that the main drawback is long extraction time. The microwave causes rapid disruption of the cell walls and shortens the duration of the extraction and increases the yield (Milutinović et al., 2015). However, there is no study available on the carotenoid extraction using vegetable oils as the solvent under the microwave conditions.

Table 2.3 The studies of carotenoid extraction using green solvents

Extraction method	Sample	Solvent	Carotenoids extracted	Conditions	Recovery	References
Conventional extraction	Tomato purée	Extra virgin, refined olive oil, refined sunflower oil and olive oil	β-carotene and lycopene	24 h, 4 °C	ND[1]	(Benakmoum et al., 2008)
Conventional extraction	Shrimp processing waste	Sunflower oil and methyl ester of sunflower oil	Astaxanthin	3h, 70 °C	Methyl ester of sunflower oil: 80% Sunflower oil: 60%	(Parjikolaei et al., 2015)
Conventional extraction	Green alga (*Haematococcus pluvialis)*	Olive oil, corn oil, soybean oil and grape seed oil	Astaxanthin	24h and 48 h, room temperature	Olive oil: 93.9% Corn oil: 89.3% Soybean oil:91.7% Grape seed oil: 87.5%	(Kang and Sim, 2008)
Conventional extraction	Shrimp (*Litopenaeus setiferus*) byproducts	FO	Astaxanthin	60 min, 60 °C	6.23 mg/100 g FO	(Pu et al., 2010)
Conventional extraction	Shrimp (*Penaeus semisulcatus*) and blue crab (*Portunus pelagicus*) wastes	Vegetable oils	Total Carotenoids	95 min, 78°C for blue crab 180 min, 40°C for shrimp waste	Sunflower oil: 0.207 ug/g for blue crab and 4.025 ug/g for shrimp waste	(Hooshmand et al., 2017)
Ultrasound-assisted extraction	Fresh carrot	Sunflower oil	β-carotene	20 min, 40°C, 22.5 W/m^2	334.75 mg/L sunflower oil	(Y. Li et al., 2013)
Ultrasound-assisted extraction	Peach palm fruit (Bactris gasipaes) by-products	Sunflower oil	Total carotenoids	30 min, 30°C, 1528 W/m^2	163.47 mg/100 g dried peel	(Ordóñez-Santos et al., 2015)
Ultrasound-assisted extraction	Pomegranate waste	Sunflower oil and soy oil	Total carotenoids	30 min, 51.5°C, amplitude level: 0.10	Soy oil: 85.7% Sunflower oil: 93.8%	(Goula et al., 2017)

[1]No data

2.4 Lipid Oxidation

Lipid oxidation is one of the major chemical reaction limiting shelf-life and quality of the oils. It is a degradation process that occurs in double bonds in unsaturated fatty acids, leading to the development of various oxidations and occurrence of degradation reaction products such as aldehydes, ketones, acids and alcohols. Lipid oxidation affects the aroma, taste, nutritional value and overall quality of oils (Ahmed et al., 2016).

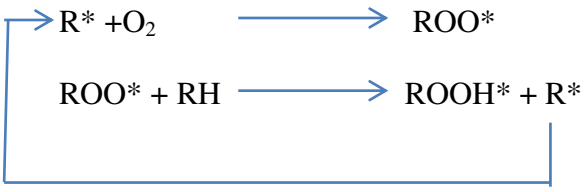
Lipid oxidation is also called auto-oxidation. This is mainly due to the ability of oils or fats with some degree of unsaturation to react with oxygen in the air, often without the need for any external catalysts. Double bonds in the unsaturated fatty acids form free radicals by oxidizing in the presence of heat, light, metal, oxygen or enzymes. The free radicals are the groups with unpaired electrons. They are extremely unstable and can easily react with the organic molecules to form more stable products. Since PUFAs contain double bonds, they are more prone to oxidation than saturated fatty acids. This is attributed to the ability of double bonds to attract electrons from neighboring carbons, making the hydrogen on the carbon atom more susceptible to abstraction and thus free radical formation (Vieira et al., 2015).

In general, auto-oxidation is a chain reaction consisting of three stages: initiation, propagation and termination (Frankel, 2014):

1. Initiation phase;

A hydrogen atom is removed from an unsaturated triglyceride molecule to form a free radical (R*).

2. Propagation phase;



The resulting free radical (R*) reacts rapidly with molecular oxygen (O_2) to form the peroxyl radical (ROO*). This radical reacts with another unsaturated lipid molecule

(RH) to form hydroperoxides (ROOH) and new free radicals (R*). In this step, the regenerated alkyl free radical reacts with molecular oxygen again and this cycle continues. As a result of the repetition of the reaction, the formation of hydroperoxides gradually increases. The primary degradation products resulting from lipid oxidation are hydroperoxides. However, hydroperoxides are not stable substances and decompose to secondary degradation products such as aldehydes, ketones, alcohols and acids. Secondary products are the main factors that cause the change of flavor, taste and odor of oils (Frankel, 2014; Ahmed et al., 2016).

3. Termination phase;

$$R^* + R^* \longrightarrow RR$$

$$R^* + ROO^* \longrightarrow ROOR$$

$$ROO^* + ROO^* \longrightarrow ROOR + O_2$$

The resulting two radicals react with each other to form non-radical products and thus the free radical chain ends. Antioxidants can react with free radicals, producing stable compound and terminating free radical chain reaction (Frankel, 2014; Ahmed et al., 2016).

Lipid oxidation must be minimized to preserve oils or fats. For this aim, oils rich in PUFAs are generally encapsulated in a polymeric matrix to minimize oxidation and to provide a long shelf-life for oils.

2.5 Encapsulation

Encapsulation is the process that active agent (core material) is entrapped in a wall material in order to maintain the functional, biological and physicochemical properties of the core materials and to avoid the chemical and physical reactions (Bakry et al., 2016).

The encapsulating substance is often referred as the carrier material, membrane, wall material, coating, matrix, shell or external phase. On the other hand, the substance that is encapsulated is also known as the core material, internal phase, fill or payload phase (Zuidam and Nedovic, 2010). Figure 2.4 presents the schematic diagram of a microcapsule demonstrating core and wall materials.

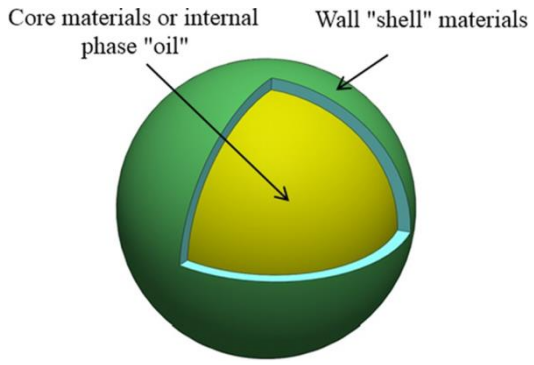


Figure 2.4 Encapsulation of oil (Bakry et al., 2016)

Generally, wall material and the encapsulation method play an important role in size and shape of the capsules formed (Bakry et al., 2016). After encapsulation process, the powder with maximum retention of the core material and minimum surface oil is considered as a successful microencapsulation (Carneiro et al., 2013).

Widely used microencapsulation methods are SD, freeze drying, coacervation, emulsification, fluidized-bed-coating, spinning disk, extrusion, liposomes, supercritical fluid technology, cocrystallization and SFD. Table 2.4 provides a brief overview of widely used encapsulation methods. SD and SFD methods used in the thesis are explained in detail below.

2.5.1 Encapsulation Methods

2.5.1.1 Spray Drying

SD is one of the oldest drying methods for the encapsulation of active agent. It has been applied in many areas, including the pharmaceutical, food, ceramic, chemical, polymer industries (Vehring et al., 2007). Figure 2.5 shows the schematic diagram of a spray dryer.

Table 2.4 Overview of common microencapsulation methods (Zuidam and Nedovic, 2010; Tavares et al., 2014; İşleroğlu et al., 2018)

Technique	Process steps	Particle size (μm)	Morphology	Examples of applications
Spray drying	1. Dissolution or dispersion of core material in aqueous coating solution 2. Atomization 3. Dehydration	10–400	Matrix	Encapsulation of flavor compounds, vitamins, polyphenols and oils
Spray-chilling/ cooling	1. Dissolution or dispersion of core material heated lipid solution 2. Atomization 3. Cooling	20-200	Matrix	Encapsulation of flavor compounds, vitamins, minerals and probiotics
Spray freeze drying	1. Dissolution or dispersion of core material in aqueous coating solution 2. Atomization in a cryogenic liquid 3. Drying under low pressure	0,2-1,400	Matrix	Encapsulation of fatty acid, peptides and pharmaceutical compounds
Coacervation	1. Preparation of oil in water emulsions with lipophilic active agent in oil phase 2. Mixing under turbulent conditions 3. Inducing three immiscible phases 4. Cooling	10-800	Reservoir	Encapsulation of fatty acids and flavonoids
Freeze drying	1. Dissolve or disperse active agent and carrier material in water 2. Freezing the sample 3. Drying under low pressure	20-5,000	Matrix	Encapsulation of fatty acids, flavor compounds and probiotics
Melt extrusion	1. Melt the coating 2. Dissolution or dispersion of core material in melted coating 3. Extruding using twin-screw extruder 4. Cooling	300-5,000	Matrix	Encapsulation of flavor compounds

Table 2.4 cont'd. Overview of common microencapsulation methods (Zuidam and Nedovic, 2010; Tavares et al., 2014; İşleroğlu et al., 2018)

Technique	Process steps	Particle size (μm)	Morphology	Examples of applications
Inclusion complexation	1. Mixing core material, coating material and water 2. Incubation and drying (if necessary)	0.001– 0.01	Molecular inclusion	Encapsulation of vitamins, flavor compounds and essential oils
Liposomes	1. Dispersion of lipid molecules in water, with an active agent in water or lipid phase 2. Size reduction using by extrusion or high shear	10-1,000	Various	Encapsulation of vitamins, enzymes and peptides
Fluid bed coating	1. Fluidizing active powder 2. Spray coating 3. Dehydration or cooling	5-5,000	Reservoir	Encapsulation of acidulates, vitamins and cells
Extrusion	1. Dissolution or dispersion of core material in alginate solution 2. Dropping into gelling bath	20-5,000	Matrix	Encapsulation of flavor compounds, vitamins and food ingredients (lactic acid)
Rapid expansion of supercritical fluid	1. Creation of a dispersion of active compound and dissolved or swollen shell material in supercritical fluid 2. Releasing the supercritical fluid to precipitate the shell onto the active compound	10-400	Matrix	Encapsulation of heat-sensible cores as vitamins and polyphenols

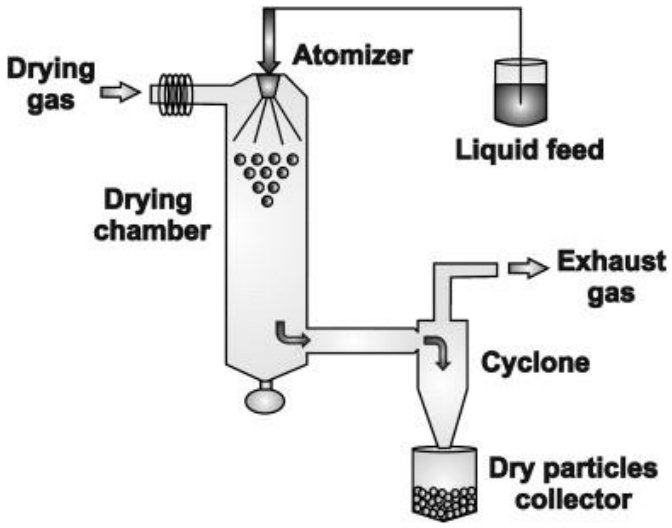


Figure 2.5 Diagram of the equipment and process of conventional spray drying (Sosnik and Seremeta, 2015)

SD process consists of four main steps (Murugesan and Orsat, 2012):

1. Atomization of the fluid feed,

2. Contact of spray with the hot air in drying chamber,

3. Evaporation of moisture,

4. Separation of product from air.

The efficiency of drying process and the encapsulated product properties depend on four phases mentioned above and their operational parameters. The feed solution can be in the form of a solution, a suspension or a paste. Process operating parameters and the physicochemical properties of the feed solution have a significant impact on the form of the encapsulated product which can be in the form of powders, granules and agglomerates (Murugesan and Orsat, 2012).

A centrifugal wheel (also called rotary atomizer) or high pressure nozzle is usually used for atomizing the feed in spray-dryers. They employ with a co-current flow of particles and air, resulting in minimum overheating of the particle. This type of flow is especially critical for the compounds which are volatile (e.g. aromas) or heat sensitive. However, the particles obtained using by co-currently flow mode are likely to be more porous than ones obtained using by the counter-current flow mode (Zuidam and Nedovic, 2010).

The velocity of the spray, the viscosity and surface tension of the liquid and pressure drop across the nozzle affect the particle size of the droplets atomized. The droplet size of the particle during atomization also determines the particle size of the powder product and drying time (Mishra, 2015).

A film is formed at the droplet surface during the drying process and thus the ingredient concentrations in the drying droplet rise. At the end of the drying, a porous, dry particle is formed (Zuidam and Nedovic, 2010).

The operational conditions of SD must be optimized in order to obtain the products with high microencapsulation efficiency. Air inlet temperature, air outlet temperature and feed temperature are the major factors in SD that must be optimized. Air inlet temperature is directly proportional to the final water content and the drying rate of the encapsulated powder. A low air inlet temperature results in low evaporation rate. This leads to the formation of encapsulated powder that has high water content, poor fluidity and high density membranes. Also, the encapsulated powder obtained is more prone to agglomeration. On the other hand, when the air inlet temperature is high, an excessive evaporation causes cracks in the membrane inducing subsequent premature release and a loss of volatiles or a degradation of active agent encapsulated. In general, two factors are considered when determining the air inlet temperature: the temperature which can be safely used without creating operating hazards or damaging the product and the comparative cost of heat sources (Gharsallaoui et al., 2007). The inlet air temperature generally ranges from 150–220^{o}C for SD process (Mohammed et al., 2020).

The air outlet temperature is the temperature at the end of the drying zone and depends on the drying conditions. It can be considered as the control index of the dryer. However, predicting the outlet temperature is quite difficult because it depends on the drying characteristics of the material to be encapsulated. Unlike the air inlet temperature, direct control of the air outlet temperature is not possible since it also depends on the air inlet temperature. Table 2.5 summarizes the experimental conditions for the encapsulation of various types of oils by SD.

Table 2.5 Optimization of spray drying process conditions for various types of oils

Encapsulated oil	Wall Material	Total Solids	Inlet/Outlet Temperature	Reference
Nigella sativa oil	Maltodextrin + Sodium octenyl succinic starch	25%	140°C/95°C	(Abedi et al., 2016)
Walnut oil	SMP + Tween 80	30%	180°C /NM	(Shamaei et al., 2017)
Rice bran oil	Jackfruit seed starch + Whey protein isolate	30%	140, 150 and 160°C	(Murali et al., 2017)
Palm fibre oil	Gum Arabic	20–40%	130–20°C /NM	(Carmona et al., 2018)
Corn oil	Brea gum	30–40%	150°C /60°C	(Castel et al., 2018)
Squash seed oil	Maltodextrin + Gum Arabic	25, 30 and 35%	140, 160, 180 ◦C/ 90°C	(Pino et al., 2019)
Lavender oil	Maltodextrin + Gum Arabic	25, 30 and 35%	140°C /95°C	(Burhan et al., 2019)
Fish oil	Whey protein	30%	160°C /NM	(Lavanya et al., 2019)
Echium oil	Gum Arabic	30%	150°C /NM	(Comunian et al., 2019)
Almond oil	Isolated starch	30–40%	145°C /NM	(Hoyos-Leyva et al., 2019)
Gac peel oil	Whey protein + Gum Arabic	24.5%	160°C /NM	(Chuyen et al., 2019)
Citronella oil	Gum Arabic	20–60%	136–203°C	(Yingngam et al., 2019)
Pomegranate Seed Oil	Xanthan gum + Gum Arabic	30, 35 and 45%	170 ◦C/85°C	(Yekdane and Goli, 2019)
Rapeseed oil	Soy protein isolate + Maltodextrin	30%	140–220°C /NM	(Linke et al., 2019)
Sour cherry oil	Maltodextrin + Gum Arabic	16.59–33.41%	120–220°C /NM	(Başyiğit et al., 2020)
Virgin coconut oil	Soy protein isolate + Maltodextrin	20 to 30%	160 and 180°C	(Quispe et al., 2020)
PUFA-rich vegetable oil	Maltodextrin + Modified starch	2:1 (wall: oil)	150 and 180°C	(Vélez-Erazo et al., 2020)

NM: non mentioned

The total solids concentration calculates and represents on a dry basis. It refers to the (oil + wall material) ratios in the emulsion. In general, the increase in total solid is desirable as it causes an increase in viscosity of the emulsion, which results in a reduction of internal circulation of the droplets and in formation of a rapid film. This can improve encapsulation efficiency and retention of active agent. However, too high a wall solid content which increases the viscosity of the feed emulsion is not favorable. The atomization process can be hindered by high emulsion viscosity, thus causing lower drying rate, larger droplet sizes and larger particle size. The size of the encapsulated product is important as it affects functional characteristics of the product such as encapsulation efficiency, targeted delivery in the appropriate functional site and controlled release. Thus, total solid content should be optimized according to desired properties of microcapsules (Anandharamakrishnan and Ishwarya, 2015; Selvamuthukumaran, 2019).

2.5.1.2 Spray Freeze Drying

SFD is relatively a new drying technique that produces powdered products with the signature product characteristics. It has been recently used in the pharmaceutical and the food industries. SFD is a unique technique which combines aspects of both freeze drying and SD. Thus, products encapsulated by SFD show the characteristics of both, namely a porous microstructure (as from freeze drying) and a free flowing powder (as from SD) (Anandharamakrishnan and Ishwarya, 2015).

One of the first reports on SFD was performed by Malecki et al. (1970) to attempt the reduce in drying time of a conventional freeze drying process by atomizing a liquid feed into a suitable cryogenic solvent such as liquid nitrogen, maintaining low temperature zone. Following this, the resulting frozen particles were sublimated under atmospheric pressure. Logically, heat transfer and mass transfer can be enhanced by the reduction in product dimensions and therefore, freezing and freeze drying times reduce (Vishali et al., 2019).

SFD is a unique drying technique which involves three operation steps (Anandharamakrishnan and Ishwarya, 2015):

1. Droplet formation by atomization: atomization generates fine droplets of liquid feed, resulting in a large air-liquid interfacial area which promotes rapid freezing

on contact with a cryogen such as liquid nitrogen at ultra-low temperature. Two-fluid, three-fluid, four-fluid and ultrasonic nozzles have been generally used for the atomization process. Among different types of nozzles used, four-fluid nozzle was reported to be suitable for drugs with poor aqueous solubility and ultrasonic nozzle was found to have good control over the particle size. Other than this, atomization process plays an important role in determining the particle size distribution of the sprayed droplets. Atomization energy, feed viscosity, surface tension and the feed flow rate are the major factors affecting atomization process (Anandharamakrishnan and Ishwarya, 2015; Vishali et al., 2019).

2. Freezing: After atomization, freezing is carried out below the sub-zero temperatures by using a cryogenic solvent such as liquid nitrogen. Freezing concentrates the core compound in the wall matrix by solidifying the solvent (often water) into tiny ice crystals. Freezing rate determines the size of the resulting solidified droplets (Anandharamakrishnan and Ishwarya, 2015).
3. Sublimation by a freeze dryer: The solidified droplets formed in the earlier step are sublimated by a freeze drying which operates at a very low temperature and pressure. As the frozen ice crystals sublimate from the droplets, they leave little pores and gaps within the encapsulated product. However, particles maintain the spherical shapes obtained from the spraying step due to rapid freezing process during SFD (Anandharamakrishnan and Ishwarya, 2015; Vishali et al., 2019).

2.5.1.2.1 Classification of SFD Approaches

The SFD is categorized based on the process variables involved in the spray freezing and freeze drying process. The physical state of the cryogen used for freezing action is the focus with respect to spray freezing. There are a number of different methods for spray freezing. They can be classified as follows. Also, the studies using these different SFD approaches are summarized in Table 2.6.

1. Spray freezing into vapour: this process involves atomizing a feed solution using by a spray nozzle and contacting the sprayed droplets with the cold desiccated gas. As a result, frozen droplets are formed. Freezing rate and nucleation rate are the most important factors which affect the microstructure of frozen droplet during spray freezing (Ishwarya et al., 2015; Vishali et al., 2019).

2. Spray freezing into vapour over liquid: in this process, a feed solution is atomized through a spray nozzle which is positioned at a small distance just above a boiling cryogenic liquid. Droplets begin to solidify in the vapour gap and then they get completely frozen when they contact the cryogenic liquid. The atomization process is crucial and affects the characteristics of the encapsulated powder obtained. The major disadvantage of this technique is the instability of biological products due to protein adsorption at the ice-water interface. (Ishwarya et al., 2015). Figure 2.6 shows schematic diagram of spray freezing into vapor over liquid.

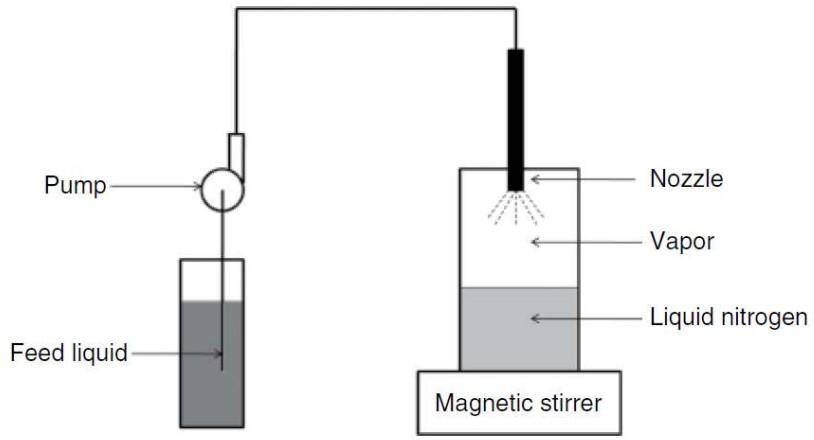


Figure 2.6 Schematic diagram of spray freezing into vapor over liquid (Anandharamakrishnan and Ishwarya, 2015)

3. Spray freezing into liquid: in this process, liquid feed is atomized beneath the surface of a cryogenic liquid such as liquid nitrogen, pentane, argon or hydrofluoroethersthe. The spray droplets start to freeze immediately after they are formed. In order to avoid clumping of the particles, the cryogenic liquid might be stirred by an impeller inserted into the vessel. The final dry and free flowing powders are obtained after the sublimation of the frozen droplets (Ishwarya et al., 2015). As compared to the spray freezing into vapour method, smaller droplets can be produced by using this method (Vishali et al., 2019). Figure 2.7 shows schematic diagram of spray freezing into liquid.

Table 2.6 Selected applications of spray freeze drying

Active compound	Cryogen	Nozzle type	Air pressure/ air flow rate	Feed rate	References
Insulin	LN_2	PEEK nozzle	5000 psi (34.5 MPa)	NA	(Rogers et al., 2002)
Danazol Carbamazepine	LN_2	PEEK nozzle	4000 psi	11 mL/min	(J. H. Hu et al., 2002)
Trehalose	LN_2	Ultrasonic nozzle (120 kHz)	NA	3 mL/min	(Sonner et al., 2002)
Darbepoetin Alfa	Frozen ethanol with a LN_2 overlay	Ultrasonic nozzle (20 kHz)	NA	0.5 mL/min	(Burke et al., 2004)
Darbepoetin Alfa	LN_2	Ultrasonic nozzle (20 kHz)	NA	0.5 mL/min	(Nguyen et al., 2004)
Bovine serum albumin	LN_2	PEEKa nozzle	5000 psi	12 mL/min	(Yu et al., 2004)
Liposomal Ciprofloxacin,	LN_2	Two-fluid nozzle	NA	NA	(Sweeney et al., 2005)
Lysozyme (50 mg/mL) Lysozyme (100 mg/mL)	Isopentane	PEEK nozzle	17.2 MPa	10 mL/min	(Engstrom et al., 2007)
BSA (5 mg/mL) Lysozyme (5 mg/mL) Lysozyme (50 mg/mL) Lysozyme (100 mg/mL)	LN_2	PEEK nozzle	17.2 MPa	10 mL/min	(Engstrom et al., 2007)
Whey protein	Nitrogen gas at −10 °C, −15 °C and −30 °C	Hydraulic nozzle	8 bar	0.0125 kg s^{-1}	(Anandharamakrishnan et al., 2010)
Ovalbumin (white albumin of chicken egg)	LN_2	Two-fluid nozzle	100 kPa	25 mL/min	(Yeom and Song, 2010)

Table 2.6 cont'd. Selected applications of spray freeze drying

Active compound	Cryogen	Nozzle type	Air pressure/ air flow rate	Feed rate	References
Lactobacillus paracasei	LN_2	Pneumatic nozzle	(a) 0.15 (b) 0.3 and (c) 0.8 mL/min	2.12 and 4.52 L/min 3.08	(Semyonov et al., 2010)
Influenza subunit vaccine powder	LN_2	Two-fluid nozzle	5 mL/min	700 L/h	(Saluja et al., 2010)
Polymeric nanoparticle aggregates	LN_2	Two-fluid nozzle	0.24 L/h	240 L/h	(Cheow et al., 2011)
Bovine Serum Albumin (BSA), Mannitol, Lysozyme	LN_2	Ultrasonic nozzle (40 kHz)	NA	0.5 mL/min	(D'Addio et al., 2012)
Docosahexaenoic Acid	LN_2	Twin fluid nozzle	24 psi	30 ml/min	(Karthik and Anandharamakrishnan, 2013)
Recombinant human epithelial growth factor	LN_2	Ultrasonic nozzle (100 kHz)	NA	1 mL/min	(Yin et al., 2014)
Lactobacillus plantarum	LN_2	Twin fluid nozzle	25 m^3/h	6 mL/min	(Rajam and Anandharamakrishnan, 2015)
Lactobacillus casei	LN_2	Two-fluid nozzle	20 kPa and 30 kPa	5 ml/min	(Her et al., 2015)
Vitamin E	LN_2	Two-fluid nozzle	NA	4 ml/min	(Parthasarathi and Anandharamakrishnan, 2016)
Polyethyleneimine	LN_2	Two-fluid nozzle	150 kPa	5 mL/min	(Okuda et al., 2018)

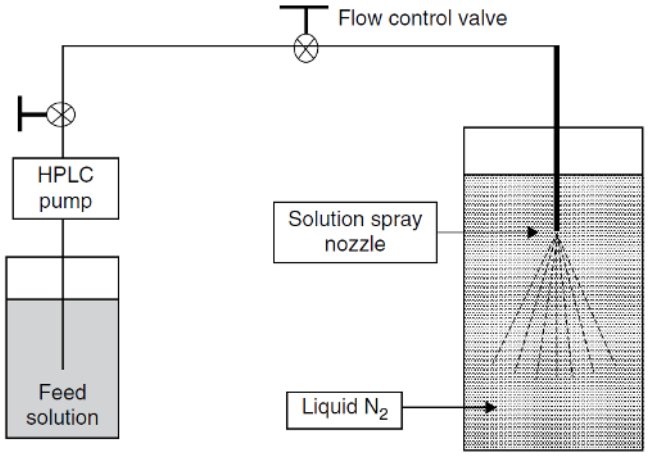


Figure 2.7 Schematic diagram of spray freezing into liquid (Anandharamakrishnan and Ishwarya, 2015)

SFD at atmospheric pressure can produce particles in the size range of 10–30 μm. Encapsulated powders are spherical shape with free flowing properties. Importantly, no phase change occurs inside the droplet between ice and the frozen eutectic liquid. SFD is considered as the gentlest process for encapsulation of heat-sensitive substances since the process is executed at low temperature.

2.5.2 Wall Materials

The selection of appropriate wall material is the most important step in the encapsulation of any core material as it influences the encapsulation efficiency and the microcapsule stability. Selected wall material for the encapsulation should form a continuous thin film and preserve the core material from deterioration. The physical and chemical properties of wall materials such as molecular weight, film forming, solubility and emulsifying properties should be considered when selecting the wall material (Gharsallaoui et al., 2007). An ideal wall material should display the following characteristics (Desai and Jin Park, 2005):

1. Chemical nonreactivity with the active core materials.
2. Easy workability during encapsulation and good rheological properties at high concentration.
3. The ability to disperse or emulsify the active material and stabilize the emulsion produced.

4. The ability to completely release the solvent or other materials used during the process of encapsulation under drying or other desolventization conditions.
5. The ability to seal and hold the active material within its structure during processing or storage.
6. The ability to provide maximum protection to the active material against environmental conditions (e.g., oxygen, heat, light, humidity).
7. Solubility in solvents acceptable in the food industry (e.g., water, ethanol).
8. Good encapsulation ability.
9. Food-grade status and inexpensive.

In the encapsulation of oils, synthetic or natural polymers are generally used as wall materials (usually polysaccharides and proteins). For the encapsulation of oils, suitable wall materials should be used to obtain the products with high stability properties. Moreover, wall materials used in the encapsulation should tend to form a thin and dense internal network during drying, and not allow oil to separate from the emulsion (Gharsallaoui et al., 2007).

Some common wall materials used in the encapsulation are explained in detail below.

2.5.2.1 Gums

Gums are generally used in encapsulation process for both their emulsion stabilization properties and film forming. Among all gums, gum arabic is the most often used gum as a coating agent due to its excellent emulsification properties. Gum arabic contains d-glucuronic, acidd-galactose, l-arabinose, l-rhamnose, and protein (approximately 2%). The presence of this protein fraction associated with gum arabic plays an important role in the emulsification properties of gum arabic (Gharsallaoui et al., 2007). Gum arabic is a surface active agent and usually preferred for encapsulation of lipid droplets as it produces stable emulsions. It fulfills the role of drying matrix and also forms a visible film at the oil interface, thus preventing the loss of volatiles in contact with the atmosphere (Madene et al., 2006; Gharsallaoui et al., 2007). However, the use of gum arabic for the encapsulation is restricted due to limited supply, expensive cost and quality variations (Gharsallaoui et al., 2007). Therefore, there is a need to evaluate alternative microencapsulation materials.

2.5.2.2 Proteins

Proteins are the good coating agents for the encapsulation by SD due to their excellent functional properties. Moreover, high binding properties of proteins makes them suitable for use in encapsulation of the flavor compounds. Whey proteins and gelatin are the most commonly used proteins for the encapsulation of food ingredients by SD (Gharsallaoui et al., 2007).

Whey proteins possess the functional properties such as efficient emulsification, gel and film forming properties that are desired for a wall material. Thus, it efficiently entraps volatile and non-volatile compounds in its polymeric matrix. Moreover, whey proteins have antioxidant activity which can prevent oxidation of the lipophilic core materials. The antioxidant abilities of whey protein are attributed to chelation of prooxidative metals and the formation of thick viscoelastic films at the droplet's oil-water interface. However, while using whey proteins as a wall material, the change in pH of the emulsion system should be taken into account as the unfolding of protein molecules at the emulsion droplet interfaces may occur due to the undesirable alterations in pH of the emulsion (Anandharamakrishnan and Ishwarya, 2015).

Gelatin is a combination of peptides and proteins which obtained by partial hydrolysis of collagen (Anandharamakrishnan and Ishwarya, 2015). It is especially preferable in the microencapsulation by SD due to its wall-forming ability and high water-solubility. In addition, gelation exhibits high emulsifying activity, high stabilizing activity and the formation of a fine dense network upon drying (Gharsallaoui et al., 2007; Anandharamakrishnan and Ishwarya, 2015). Low cost, biodegradability and non-toxic properties are the other advantages of gelatin (Anandharamakrishnan and Ishwarya, 2015).

2.5.2.3 Maltodextrin

Maltodextrin is a hydrolyzed starch obtained by acidic or enzymatic reactions. It comprises D-glucose units linked primarily by alpha-1,4 glycosidic bonds (Figure 2.8). The degree of hydrolysis of a polymer is characterized by the dextrose equivalent (DE). DE of a maltodextrin is the most important parameter which influences its functionality as a wall material. Maltodextrin exhibits the high water solubility (> 75%) and the low viscosity (Anandharamakrishnan and Ishwarya,

2015). It is widely used in the encapsulation of oils as the coating agent because it provides good oxidative stability to encapsulated oil (Gharsallaoui et al., 2007; Medina-Torres et al., 2016). It has advantages such as low cost, lack of specific aroma and flavor, low viscosity and providing a good protection against oxidation. Despite all these advantages, maltodextrin exhibits poor emulsifying capacity, emulsion stability and thus low oil retention. To improve efficiency of encapsulation, maltodextrin is generally used together with surfactant polymer such as pectin, gum arabic, modified starch and proteins (Krishnan et al., 2005; Fernandes et al., 2008; Sansone et al., 2011).

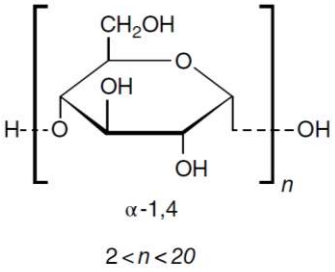


Figure 2.8 The chemical structure of the maltodextrin (Anandharamakrishnan and Ishwarya, 2015)

2.5.2.4 Pectin

Pectin is a complex polysaccharide which consists of linear chains of D-galacturonic linked α-1,4 glycosidic bonds (Bush, 2014). Figure 2.9 shows basic structure of pectin.

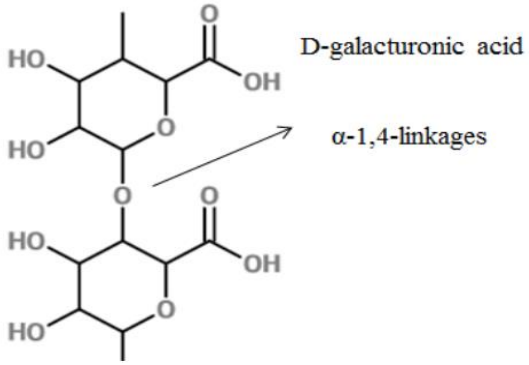


Figure 2.9 Basic structure of pectin

Pectin is one of the most widely known polysaccharides found in the cell wall of many plants. There are two types of pectin, namely low methoxy pectin and high

methoxy pectin. The structural difference between these pectins is due to the amount of esterified carboxyl groups (Ruiz and Segura-Campos, 2017). Low-methoxy pectin possesses a degree of esterification of less than about 50%. On the other hand, pectins with more than 50% of the carboxyl groups esterified are called as high methoxy pectin.

Pectin can be used as an alternative to expensive wall materials such as gum arabic and proteins because it is low cost (Drusch, 2007). It is a polymer able to produce stable emulsions at low concentration. The emulsifying activity of pectin is related to its protein portion within the pectin chain and the presence of acetyl groups (Leroux et al., 2003).

There are some studies on the use of sugar beet pectin as a wall material for encapsulation of lipophilic food ingredients by SD. Drusch (2007) reported that fish oil was successfully encapsulated using sugar beet pectin in the formulation. They suggested that sugar beet pectin is a suitable wall material for SD. Another study conducted by Leroux et al. (2003) observed that a 2% concentration of the pectin solution had an effect similar to a 15% concentration of gum Arabic. Ghasemi et al. (2017) used two different biopolymers, pectin and whey protein, for producing orange peel oil-loaded nanocapsules. They concluded that pectin and whey protein mixture was a suitable wall material for encapsulating orange peel oil due to its high encapsulation efficiency. Moslemi et al. (2014) used pea protein and pectin mixture for encapsulating PUFA-rich oil. After encapsulation by SD, the resultant powder was characterized by encapsulation efficiency of 77.52% and better oxidative stability. Also, pectins extracted from pumpkin and citrus might be used for encapsulating polyphenols by SD process (de Souza et al., 2013).

2.5.2.5 Wax

Waxes, long-chain esters of alcohol and fatty acids, are commonly used as an edible coating to improve the water vapor and oxygen barrier properties of food products (Chick and Hernandez, 2002; Bourlieu et al., 2009). They can naturally be found in seeds and fruits. They are an important by-product of the refining process of several vegetable oils (Baumler et al., 2013). They have been used as carrier or coating material in pharmaceutical applications (Mellema et al., 2006). The use of waxes as an encapsulation material is especially important in systems that require protection

against moisture and oxygen. In a study examining the physical properties of pectin solution containing wax, it was reported that pectin-wax mixture as a coating material has a great potential to extend shelf life of foodstuff (Baumler et al., 2013). Lozano-Grande et al. (2016) produced edible films using hawthorn (*Crataegus* spp.) fruit pectin and candelilla wax and evaluated their potential usage as a coating material for fresh fruits. They reported that pectin-wax coating material has low water vapor permeability and thus can be a good barrier to a water vapor exchange.

CHAPTER 3

MATERIALS AND METHOD

3.1 Chemicals and Reagents

All chemicals and reagents used in this study were of analytical or high performance liquid chromatography (HPLC) grade. Standard of β-carotene Type II (≥95% purity by HPLC assay), folin–ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), sodium chloride (NaCl), ascorbic acid, 1,1-diphenyl-2- picrylhydrazyl (DPPH), potassium hydroxide (KOH), 1,1,3,3-tetraethoxypropane were purchased from Sigma-Aldrich. Gallic acid was provided by Merck.

3.2 Materials

CPW used as a source of carotenoid in the experiments was supplied by a producer of carrot juice. The waste was frozen at -40°C prior to freeze drying and then moisture content was reduced to below 1% by using freeze dryer (CHRIST, model Alpha 1-4 LDplus, Martin Christ, Germany). The dried waste was ground in a waring blender. The resulting powder (smaller than 0.85 mm) was stored at -20°C in opaque containers until carotenoid extraction.

Flaxseed was supplied by the local market and pressed by using cold press oil machine (Karaerler, NF 100, Ankara, Turkey) to obtain the cold-pressed FO in the laboratory.

Low methoxylated pectin (LMP) (Yantai Andre Pectin Co. Ltd., China, type APA 210), sunflower wax (SFW) (Koster Keunen Holland, B.V., melting point: 74-77) and maltodextrin (MD) (Roquette - (DE) Dextrose equivalent: 6) were used in emulsion preparation.

3.3 Microwave Assisted Carotenoid Extraction (MACE) Using FO

The certain quantity of the waste powder (the quantity varied as a function of the oil to solid ratio) was weighed into the flask and mixed with FO as solvent. Total amount (oil+ solid) was kept at 20 g. Thirty five ml extraction vessel (88 mm

height and 25 mm diameter borosilicate glass with a silicone cap) was placed in the microwave extraction device and the extraction process was operated after the microwave power and extraction time were set up according to the experimental design (Table 4.1). Carotenoid extraction from the waste powder was carried out in a closed microwave system (CEM Corporation, model Discover-SP, Matthews, NC, USA). The microwave chamber has been cooled by air circulated in ice bath throughout the microwave application. The temperature did not exceed 110 ^{o}C for even the harshest experimental runs (highest microwave power and longest time). CEFO was stored in dark glass bottles at -20°C until the analyses were performed.

3.4 Conventional Carotenoid Extraction (CCE) Using FO

Approximately, 0.95 g of the waste powder was mixed with 19.05 ml of oil (Oil to solid ratio=20:1). Total amount (oil+ solid) was kept at 20g as in MACE. The mixture was heated at 65^{o}C in a mixer incubator (Heidolph, Unimax 1010, Schwabach, Germany) for 270 min. The samples were taken out at 12, 30, 60, 90, 120, 180, 240, 270 min and carotenoid contents of the samples were determined.

3.5 Soxhlet Extraction

Carotenoids in CPW were extracted by the soxhlet extraction method. Procedure with some modifications described by Bhudsawan Hiranvarachat et al. (2013) was used for the extraction of carotenoids. The waste powder (2g) was placed in an extraction thimble, which was then placed in an extraction chamber and extracted 6 h by using 150 mL of n-hexane, ethanol, acetone (2:1:1 v/v/v) solvent mixture. Solvent mixture was evaporated under vacuum at 40^{o}C using the rotary vacuum evaporator (Heidolph, type basis Hei-VAP HL, Schwabach, Germany) in order to obtain carotenoid extract. Extract was dissolved in n-hexane prior to analyses of total carotenoid and β-carotene contents. Soxhlet extraction method was used to determine the total amount of carotenoid and β-carotene content in CPW.

3.6 Total Carotenoid Content

The diluted extract obtained from soxhlet extraction was used to estimate the carotenoids content of CPW. Total carotenoid content of CPW was determined at 450 nm (absorbance maxima for β-carotene) using a spectrophotometer (Optima, model SP-3000nano, Tokyo, Japan). β-carotene standard solutions dissolved in

hexane at eight concentration (0, 4, 5, 6, 7, 8, 9 and 10µg/mL) were used to create a calibration curve at a given wavelength (450 nm) of the utilized spectrophotometer. The calibration curve prepared is shown in Figure 3.1. The total carotenoid content of CPW was expressed as µg β-carotene per g of CPW powder.

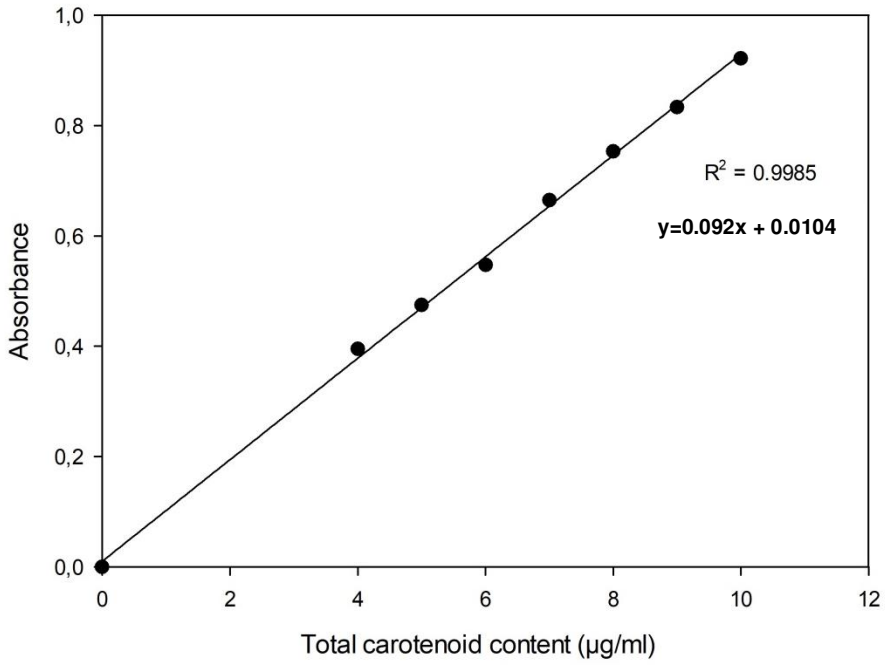


Figure 3.1 Calibration curve for total carotenoid content (β-carotene standard prepared in n-hexane)

The total carotenoid content of FO and CEFO was also determined spectrophotometrically, reading absorbance at 450 nm against FO as blank (Sachindra and Mahendrakar, 2005). Calibration curve (0, 2.5, 5, 7.5 and 10µg/mL) prepared using the β-carotene standard dissolved in FO was used to determine the carotenoid concentration in FO and CEFO. Figure 3.2 shows the calibration curve prepared.

Percent recovery of carotenoid from CPW was calculated using Equation (3.1) below.

$$\text{Recovery (\%)} = (\text{CE/CW}) \times 100 \qquad (3.1)$$

where CE is amount of carotenoid extracted and CW is total carotenoid content of CPW.

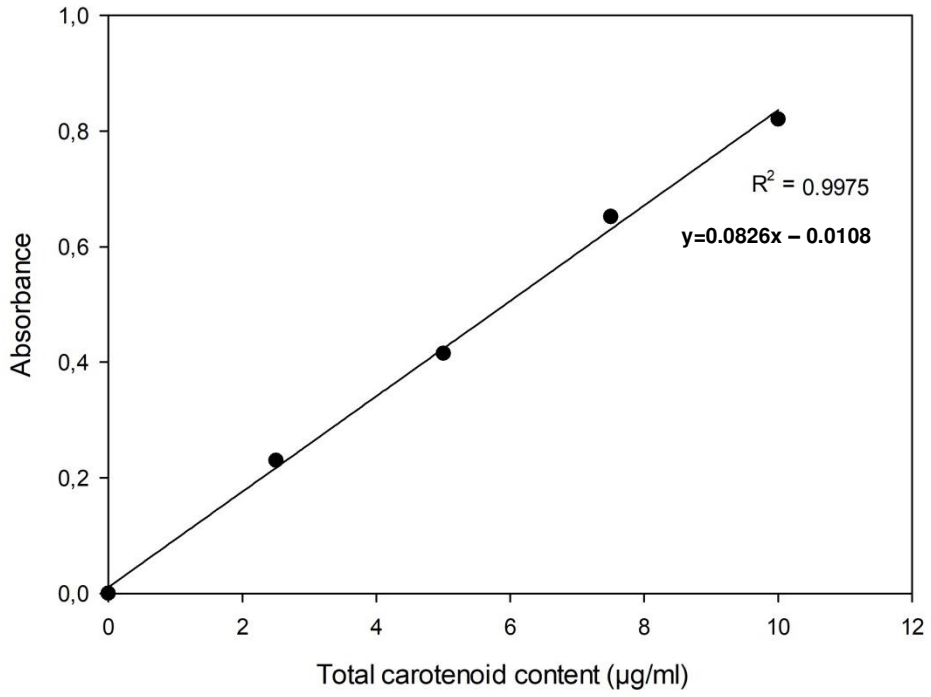


Figure 3.2 Calibration curve for total carotenoid content (β-carotene standard prepared in FO)

3.7 Experimental Design

The optimal conditions for MACE from CPW using FO as a solvent were determined by response surface methodology (RSM). RSM was performed using Design Expert, version 7 (Stat-Ease, Design-Expert software, Minneapolis, USA). Microwave power (X_1), extraction time (X_2) and oil to solid ratio (X_3) were independent variables of the system and % recovery of carotenoid (Y_1) was the response of the system. The extraction parameters were optimized through a central composite rotatable design (CCRD). The experimental design consists of 20 test points with 6 central points, 6 star points (Table 4.1).

Significances of the model and all terms were determined by evaluating the Fisher test value at a probability of 0.05. CCRD uses least-squares regression to fit the experimental data to a second-order polynomial model. The second-order polynomial equation expressed in Equation (3.2) was used to calculate predicted response.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j>1}^{k} \beta_{ij} X_i X_j \qquad (3.2)$$

where β_0, β_i, β_{ii} and β_{ij} are defined as the constant, linear, quadratic and interaction coefficients, respectively. X_i and X_j are the level of the independent variables.

3.8 Quantification of β-Carotene by HPLC

Rapid saponification was used to extract carotenoids from oil samples prior to the analysis of β-carotene. The procedure for extraction with saponification was adapted from the method by Gimeno et al. (2000).

The quantity of β-carotene using HPLC (Shimadzu, Prominence/LC-20AB, Kyoto, Japan) was determined by the method proposed by Barba et al. (2006). Mobile phase, mixture of methanol/ acetonitrile (90:10 v/v) + 9μM triethylamine, was eluted isocratically at a flow rate of 0.9 ml/min. Supelcosil LC-18 5 μm (4.6 x 250 mm) HPLC column (Supelco, Bellefonte, PA, USA) was used for β-carotene analysis. Detection of β-carotene was made at a wavelength of 450 nm using UV–vis detector (Shimadzu, SPD-20A, Kyoto, Japan). Quantification of β-carotene was performed through a calibration curve. β-carotene standard solutions at seven concentrations (0, 0.1, 0.5, 1, 2.5, 5 and 10μg/mL) were prepared daily and injected into HPLC to create the calibration curve (Figure 3.3).

3.9 Fatty Acid Composition

The fatty acid compositions of FO and CEFO were identified according to procedure proposed by Yanik (2017), using an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with a split/splitless injector, flame ionisation detector and HP-88 capillary column (88 % cianopropylaryl; 100 m×0.250 mm, 0.20 μm i.d.). The inlet temperature was 250°C. The carrier gas was hydrogen at 2.0 mL/min constant flow. The oven temperature was programmed at an initial 120°C, held for 1 min, followed by an increase of 10°C / min up to 175°C, held for 10 min, followed by an increase of 5°C /min up to 210°C, held for 5 min, followed by an increase of 5°C /min up to 230°C and held for 5 min. The detector was set at 260 °C with 350 mL/min airflow, 35 mL/min hydrogen flow, and 15 mL/min helium makeup flow. Fatty acid standards were used to identify the peaks.

3.10 Peroxide, Acid and p-Anisidine Values

The peroxide and acid values of oil samples were determined according to the method of AOCS Cd 8-53 (AOCS, 1998) and AOCS Cd 3d-63 (AOCS, 1998),

respectively. P-anisidine values of FO and CEFO were determined according to AOCS Cd 18-90 (AOCS, 1998).

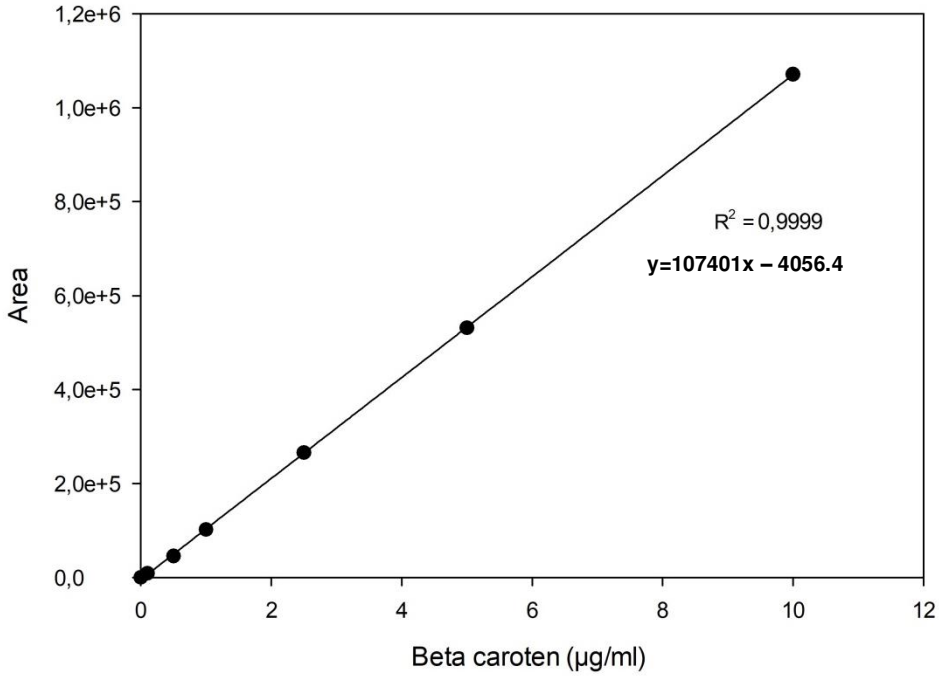


Figure 3.3 β-carotene content calibration curve (HPLC method)

3.11 Total Phenolic Content (TPC)

Phenolics of FO and CEFO were extracted following the method described by (Kiralan et al., 2009).

TPC of FO and CEFO were determined with the Folin-ciocalteu colorimetric method presented by Singleton et al. (1999) with some modification. TPC values were expressed as µg gallic acid equivalents (GAE)/ g oil.

3.12 DPPH-Scavenging Activity Assay

Antioxidant activities of FO and CEFO were determined according to the procedure with some modification reported by Kiralan et al. (2009). Inhibition (%) was calculated using the following equation:

$$\% \text{ Inhibition} = (1 - [A_{sample}/A_{blank}]) \times 100 \quad (3.3)$$

Where A_{sample} is the absorbance of sample with DPPH solution, A_{blank} is the absorbance of DPPH solution without sample solution at 517 nm.

3.13 Colour Measurement

The colours of FO and CEFO were measured using a Hunter-Lab ColorFlex (HunterLab, model A-60-1010-615, Reston, VA). The colour value was expressed as L* (lightness; from 0 = black to 100 = white), a* (negative values indicate greenness while positive values indicate redness) and b* (negative values indicate blueness and positive values indicate yellowness).

3.14 Emulsion Preparation

LMP and MD were stirred in distilled water at 300 rpm for 30 min at 80°C in order to dissolve LMP and MD completely. Solid SFW was weighed in a beaker and melted at 80°C while LMP-MD solution was prepared. Melted SFW was mixed with CEFO and then added into LMP-MD solution. All mixture was homogenized using a high shear homogenizator (IKA, T18 Digital Ultra-Turrax) at 25,000 rpm for 5 min at 80^{o}C to prevent the crystallization of wax. An example emulsion is given in Figure 3.4.

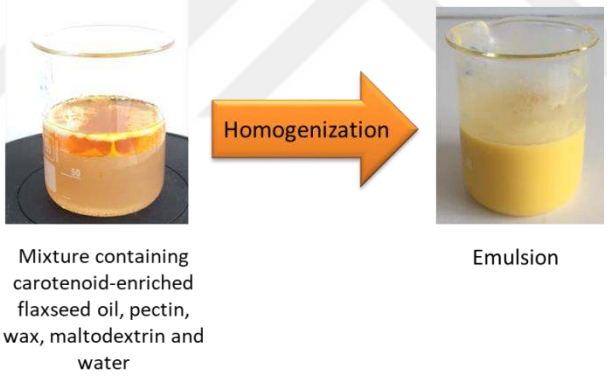


Figure 3.4 The preparation of an emulsion

The amount of wall materials (LMP + SFW + MD) and CEFO in emulsion was kept constant at 30%. LMP and SFW ratio was determined as 2:1 (g/g) by preliminary experiments and this ratio was kept constant in all runs. Experimental design for the encapsulation is given in Table 3.1.

Table 3.1 Experimental design of emulsion formulation

Oil concentration in emulsion (%)	6	9	12	15
MD/(LMP+SFW) (g/g)	3:1	3:1	3:1	3:1
	6:1	6:1	6:1	6:1
	9:1	9:1	9:1	9:1
	12:1	12:1	12:1	12:1

(CEFO: carotenoid-enriched flaxseed oil; MD: maltodextrin LMP: low methoxylated pectin SFW: sunflower wax)

3.15 Stability of Emulsion

Immediately after the emulsion preparation, 25-mL aliquots of each sample were transferred to graduated cylinders, sealed, stored at room temperature for 24 h, and the volume of the upper phase was measured after 24 h. The stability was measured by the percentage of separation using following equation.

$$\% \text{ Separation} = [(H_1) / (H_0)] \text{ x}100 \quad (3.4)$$

where H_1 is height of the upper phase and H_0 is initial height of emulsion

3.16 Rheological Measurements

The rheological behavior of the emulsions was determined in the shear rate range of 0-200 s^{-1} during 60 s at 20°C by using Haake Rheostress RS1 (Karlsruhe, Germany) equipped with TCP/P Peltier temperature controller unit. A plate-plate sensor (diameter 3.5 cm; gap 1 mm) was used throughout the measurements. The shear rate versus shear stress data was analyzed with a Power law model (RheoWin 3, Haake, Karlsruhe, Germany) according to the following equations:

$$\tau = K\dot{\gamma}^{n} \quad (3.5)$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency index ($Pa.s^n$) and n is the flow behavior index. Apparent viscosity was calculated at 50 s^{-1} of shear rate.

3.17 Particle Size Measurements

Particle size analysis was performed using a particle size analysis device (Model LA-950, Horiba, Tokyo, Japan). Briefly, a small amount of sample was dispersed in water using a magnetic stirrer and each measurement was monitored until successive readings became constant. The mean particle size of the emulsions was evaluated as the volume mean diameter (D_{43}) given in Equation (3.6).

$$D_{43} = (\sum_i z_i D_i^4)/(\sum_i z_i D_i^3) \quad (3.6)$$

where n_i is the number of droplets of diameter (d_i).

3.18 Encapsulation Methods

3.18.1 Spray Freeze Drying

Emulsions of CEFO with wall materials (100 mL) prepared according to the formulation in Table 3.4 were atomized via an ultrasonic nozzle (60 kHz frequency, 1–15 W power output at nozzle, Buchi Corporation, Flawil, Switzerland) into a 4-L ice pan full of liquid nitrogen. The atomized fine droplets were frozen in liquid nitrogen. Atomization was carried out under the following conditions; 4 mL/min (15%) of feed flow rate and 9 W of power output at nozzle. The distance between ultrasonic nozzle and liquid nitrogen was maintained at 10 cm. After freezing, the excess liquid nitrogen was removed from the slurry by evaporation, and the resultant frozen droplets were transferred to a pre-cooled shelf (~ − 45°C) of a freeze dryer (CHRIST, Alpha 1-4 LSC, Germany) for the removal of water. At the end of the drying (after 18 h), the dried encapsulated powder was stored in a brown glass bottle until the analyses were performed.

3.18.2 Spray Drying

SD was performed using a model B-290 Büchi mini spray dryer (Büchi Labortechnik AG, Switzerland). An ultrasonic nozzle (the same nozzle used in SFD) was used for the atomization. The process conditions were the following: 135°C of inlet air temperature, 9 W of power output at nozzle, 4 mL/min (15%) of feed flow rate, 30m^3/h of air flow rate and 90% of aspirator rate. After drying process, the powder product was stored in a brown glass bottle until the analyses were performed.

3.19 Encapsulation Efficiency

Surface oil content was determined with some modifications according to the method described by (Quispe-Condori et al., 2011). Briefly, 500 mg of the encapsulated powder product was weighed on Whatman no.1 filter paper. The powder product on the filter paper was washed 5 times using 1 mL hexane (5 mL in total). The filtrate solution containing extracted oil was collected in a clean glass container that was tared. After evaporation of the solvent at room temperature, it was dried in an oven at 60^{o}C until reaching constant weight. The amount of oil on the surface was determined by the weight difference before and after washing with hexane. Since FO is not a volatile oil, the total oil amount was considered to be equal to the initial oil amount.

The wax used as wall material in encapsulation is a nonpolar compound (Akoh, 2017) and therefore, some amount of wax on the surface has been extracted along with oil during washing with hexane. The wax ratio of the extracted oil-wax mixture was determined by thin layer chromatography and flame ionization detector (TLC-FID) (described in 3.20) and the amount of surface oil was corrected by subtracting the wax content. The encapsulation efficiency was calculated using the equation below. Measurements were performed in triplicate.

$$\text{Encapsulation efficiency (\%)} = [(TO-CSO) / TO] \times 100 \quad (3.7)$$

TO: Total oil content

CSO: Corrected surface oil content

3.20 Determination of Wax Content by TLC-FID

The procedure was carried out according to the method reported by Ungcharoenwiwat and H-Kittikun (2015). Latroscan device (Iatroscan MK-5; Iatron Laboratories, Tokyo, Japan) was used to separate wax and oil. Scanning was carried out under the following conditions: air flow rate = 2.0 L/min, gas flow rate = 160 mL/min, scanning speed = 30 s/scan. It is assumed that the percentage of peak area on the chromatogram is the percent content of the related compound. Measurements were performed in triplicate.

3.21 Moisture Content

The moisture content of each sample (~1 g) was determined by isothermal drying using vacuum oven at 70 °C. Measurements were performed in triplicate.

3.22 Particle Size Analysis

Particle size analysis of the powder was performed using a particle size analysis device (Model LA-950, Horiba, Tokyo, Japan). A small amount of the encapsulated powder was dispersed in absolute ethanol using a magnetic stirrer and each measurement was monitored until particle size distributions were read successfully. The mean particle size of the encapsulated powder was evaluated as volume mean diameter (D_{43}). Measurements were performed in triplicate.

3.23 Flowing Properties

The bulk density (ρ_0) and tapped density (ρ_{50}) of the encapsulated powder were determined according to the method with some modifications described by Carneiro et al. (2013). Three grams (3 g) of each sample (m_0) was poured through a funnel into a 25 mL glass graduated cylinder. Then, the cylinder was slightly tapped to collect the powder sticking to the wall of the cylinder. The volume (V_0) was read directly from the cylinder and used to calculate the bulk density ($\rho_0 = m_0/V_0$). For tapped density ($\rho_{50} = m_0/V_{50}$), the cylinder was tapped for 50 times from a height of 10 cm and tapped volume (V_{50}) was read from the cylinder.

The flowing properties of the encapsulated powders were determined by the Hausner ratio (HO) and Carr index (CI) given below. Measurements were performed in triplicate.

$$HO=\rho_{50}/\rho_0 \tag{3.8}$$

$$CI = (\rho_{50}- \rho_0/ \rho_{50})x100 \tag{3.9}$$

ρ_0: Bulk density

ρ_{50}: Tapped density

3.24 Scanning Electron Microscopy

Morphology of the encapsulated powder was obtained using Zeiss GeminiSem300 (Carl Zeiss Ltd. Co., Germany) scanning electron microscopy (SEM). The encapsulated powders were attached to aluminum stubs and coated with gold: palladium in an Emitech SC7620 (Kent, UK) sputter coater.

3.25 Oxidative Stability

Oxidative stabilities of the encapsulated powders obtained by SFD and SD methods were characterized using peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) tests. Encapsulated powders were stored within sealed nitrogen-flushed 100-mL amber glass bottles at room temperature (25^{o}C) for 45 days. Oxidative stability tests were performed every 5 days for first 25 days, and every 10 days in the following 25 days.

3.25.1 Peroxide Value

PV was determined according to the method with some modifications described by (Karaca et al., 2013) and expressed as milliequivalents (meq) of O_2 per kilogram of oil. Approximately 1 g of extracted oil was weighed into a 100 mL erlenmeyer flask, followed by the addition of 10 mL chloroform, 15 mL of acetic acid and then 1 mL of saturated potassium iodide. After shaking for 1 min, the flask was placed in a dark place for 8 min. Then, 25 mL of water and 1 mL of 1% starch indicator solution were added and finally the mixture was titrated using a 0.001 N $Na_2S_2O_3$ standard solution. Measurements were performed in triplicate. The PV was calculated as given by the following equation:

$$\text{PV (meq } O_2\text{/kg oil)} = \frac{(a-b)\times N\times 1000}{m} \qquad (3.10)$$

a= Volume of $Na_2S_2O_3$ consumed in the sample titration, mL

b= Volume of $Na_2S_2O_3$ consumed in the blank titration, mL

N= Normality of $Na_2S_2O_3$, N

m= The sample weight, g

3.25.2 Thiobarbituric Acid Reactive Substances (TBARS)

TBARS contents of encapsulated powders were calculated using the method of Pu et al. (2011). TBARS content was determined by the calibration curve prepared using 1,1,3,3-tetraethoxypropane (TEP) (Figure 3.5). Measurements were performed in triplicate.

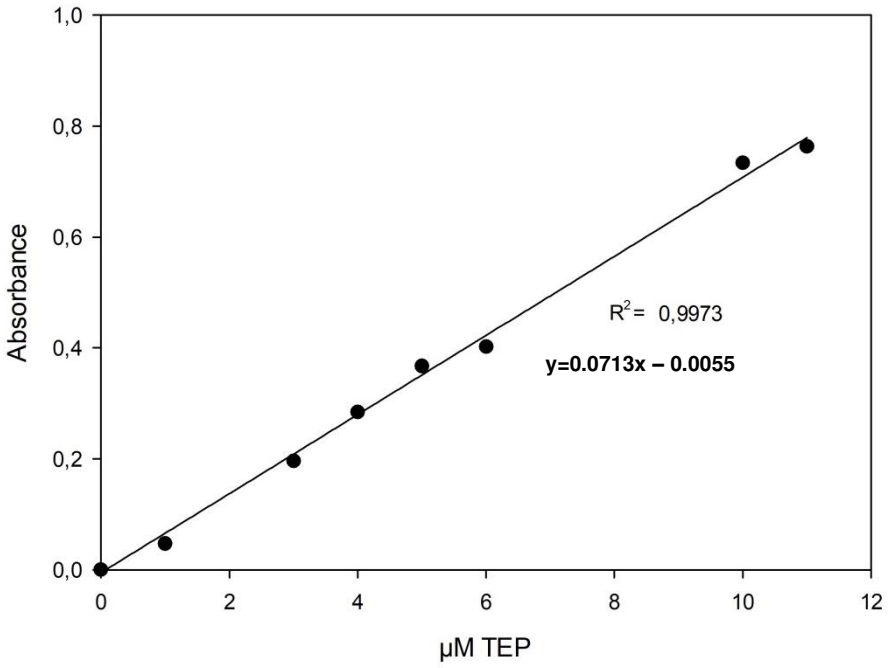


Figure 3.5 Calibration curve for TBARS

3.26 Statistical Analysis

All experiments were performed in triplicate and the results were reported as mean ± standard deviation (SD). Statistical differences were evaluated by one-way ANOVA and significant differences between results determined by Duncan's test at p=0.05.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 RSM Modeling

The most important factors influenced the recovery of bioactive compounds like carotenoids from plants are microwave power, extraction time and as well as solvent to sample ratio when microwave-assisted extraction is concerned (Mandal et al., 2007; Bhudsawan Hiranvarachat et al., 2013). Therefore, microwave power, extraction time and oil to solid ratio was studied as independent variables on extraction of carotenoid from CPW. Microwave power range was selected as 50-200 W according to the preliminary experiments. Because power above 200 W was caused some troubles such as charring of CPW. Studied extraction time ranged from 1 to 12 min. Longer extraction time did not cause the considerable extraction of the carotenoids from CPW. The range of oil to solid ratio was selected as 5:1-20:1 g/g based on the preliminary trials. Percentage recovery of carotenoids was considered as the dependent variable. Three factors- five levels CCRD was studied in a total of 20 experiments. Table 4.1 shows both the actual values of design parameters and the experimental results obtained under the defined points.

The adequacy and fitness of the model and the relationship between variables were examined using regression analysis and analysis of variance (ANOVA), respectively, and the results are given in Table 4.2. In this study, the model with high F-value (34.04) and a very small *p*-value (<0.0001) was highly significant and the lack of fit test was found to be non-significant ($p>0.05$). Thus, the model builded is sufficiently accurate for predicting the outcomes. The R^2 value of 0.9521 implies that there was a good correlation between experimental and predicted values of the response variable.

4.2 The Effect of Process Variable on Recovery of Carotenoids

According to the results of experiments carried out at the points designated by CCRD, the percentage recovery of carotenoid (Y_1) ranged from 45.95 to 77.91 % (Table 4.1). Highest Y1 value (77.91 %) was obtained in experimental run 4 under

the following conditions: microwave power of 170 W of, an extraction time of 9.46 min and oil to solid ratio of 8:1 g/g. While the microwave power (X1) and extraction time (X2) have been found as highly significantly ($p<0.001$) effected on extraction of carotenoids from carrot waste by FO, the oil to solid ratio (X_3) were found as non-significant ($p>0.05$) (Table 4.2). However, the quadratic effect of X_3, the interactive effect of X_1*X_3 and X_2*X_3 were found as statistically significant. The interactive effect of X_1*X_2 has also been found as non-significant. Predicted model equation obtained from the experimental data was formulated by removing the non-significant variables. However, some non-significant terms were not removed from the model in order to support the model hierarchy. Second-order polynomial equation in terms of codded factors obtained from CCRD model for recovery of carotenoid (%) was given as follows.

$$\text{Recovery (\%)} = 62.49 + 5.77* X_1 + 7.17* X_2 - 1.44* X_3 - 1.80*X_1*X_2 + 1.97*X_1*X_3 - 2.17*X_2*X_3 + 3.31*X_3^2 \quad (4.1)$$

Table 4.1 Experimental design for microwave extraction

Run	Microwave power (W)	Extraction time (min)	Oil to solid ratio (g/g)	Recovery %
1	80.00	3.14	8:1	53.87 ±0.23
2	170.00	3.14	8:1	63.09±0.36
3	80.00	9.46	8:1	74.98±1.20
4	170.00	9.46	8:1	77.91±0.33
5	80.00	3.14	17:1	51.94±0.07
6	170.00	3.14	17:1	69.97±0.71
7	80.00	9.46	17:1	65.30±0.41
8	170.00	9.46	17:1	75.22±0.11
9	50.00	6.30	12.5:1	50.06±0.21
10	200.00	6.30	12.5:1	73.07±1.17
11	125.00	1.00	12.5:1	45.95±0.19
12	125.00	12.00	12.5:1	71.74±0.30
13	125.00	6.30	5:1	74.46±0.66
14	125.00	6.30	20:1	67.15±0.37
15	125.00	6.30	12.5:1	63.88±0.85
16	125.00	6.30	12.5:1	61.69±0.43
17	125.00	6.30	12.5:1	63.95±0.95
18	125.00	6.30	12.5:1	64.41±0.27
19	125.00	6.30	12.5:1	61.02±0.90
20	125.00	6.30	12.5:1	65.35±0.46

All the given values are means of two (n = 2) determinations ± SD

Table 4.2 Regression analysis and analysis of variance

Source	Recovery (%)				
	SS[a]	DF[b]	MS[c]	F -Value	p-value Prob > F
Model	1440.35	7	205.76	34.04	< 0.0001[d]
Linear					
Microwave Power (X_1)	454.61	1	454.61	75.21	< 0.0001[d]
Extraction Time (X_2)	702.14	1	702.14	116.16	< 0.0001[d]
Oil to solid ratio (X_3)	28.48	1	28.48	4.71	0.0507[e]
Interaction					
X_1*X_2	25.94	1	25.94	4.29	0.0605[e]
X_1*X_3	31.16	1	31.16	5.16	0.0424[d]
X_2*X_3	37.51	1	37.51	6.21	0.0284[d]
Quadratic					
X_3^2	160.51	1	160.51	26.55	0.0002[d]
Residual	72.54	12	6.04		
Lack of Fit	58.60	7	8.37	3.00	0.1220[e]
Pure Error	13.93	5	2.79		
Cor Total	1512.89	19			
R^2	0.9521				
Adj R^2	0.9241				
Pred R^2	0.8516				

[a] Sum of Squares; [b]Degree of freedom; [c]Mean square
[d] Significant at Prob > F less than 0.05 level; [e] Not significant at Prob > F higher than 0.05 level

Based on the regression coefficient values (β), the highest influence on the recovery of carotenoids was extraction time, followed by microwave power. However, it was observed that no significant effect of oil to solid ratio was found on recovery of carotenoid. Extraction time has positive effect on the response in linear term. It means that the increase in carotenoid content extracted corresponded to the rise in extraction time. This result was in good agreement with previous study about β-carotene extraction from carrot using sunflower oil as a solvent under ultrasound treatment (Y. Li et al., 2013). It was found that β-carotene extraction yield could be increased by increasing the time. In another study carried out by Goula et al. (2017), it was reported that the extraction yield was time dependent and increased with extended ultrasonic times, especially from 10 to 30 min. Microwave power also showed a significant (p<0.0001) positive linear effect on carotenoid extraction (Table 4.2). From Figure 4.1, it is understood that microwave power had a strong sway on MACE. Microwave energy absorbed by materials depends on their dielectric properties (Regier et al., 2017). Water, which has a large electric dipole moment, is very efficient at absorbing the microwave energy. On the other hand, oils are esters of long-chain fatty acids which have much less mobility compared to water molecules in response to oscillating microwave irradiation. Therefore, they have

rather small dipole moments and don't absorb the radiation as much as water. However, the specific heat of oils is sufficiently smaller than the specific heat of the water, and then oils tend to heating up much faster than water when exposed to the same amount of microwaves (Cullen et al., 2012). The temperature rises of the mixture due to the increase of microwave power reduce the viscosity of the solvent (FO) and increase the solubility of carotenoids (Bhudsawan Hiranvarachat et al., 2013). This results in an enhanced diffusion rate, thereby prominent accelerating extraction rate. Even though no significant effect of oil to solid ratio was observed under microwave extraction using oil as solvent, the interaction between extraction time and oil to solid ratio showed a synergistic effect on amount of the carotenoid extracted (p<0.05). As shown in Figure 4.1B, extraction yield of the carotenoid increased with longer extraction time with low ratio.

4.3 Experimental Validation of Optimized Conditions

Microwave extraction system was optimized employing RSM. The optimum conditions were determined by a quadratic model as 165 W of microwave power, 9.39 min of extraction time, 8.06:1 g/g of oil to solid ratio to obtain optimum value of recovery (77.87%). The carotenoid extractions (three times) were performed at optimum conditions to validate the suggested model. The experimental results gave 77.48 ± 0.16 % recovery of carotenoids which was close to the predicted value. The experimental data confirmed the validity and the accuracy of predictive model. The original FO and the CEFO obtained in optimum conditions are shown in Figure 4.1.

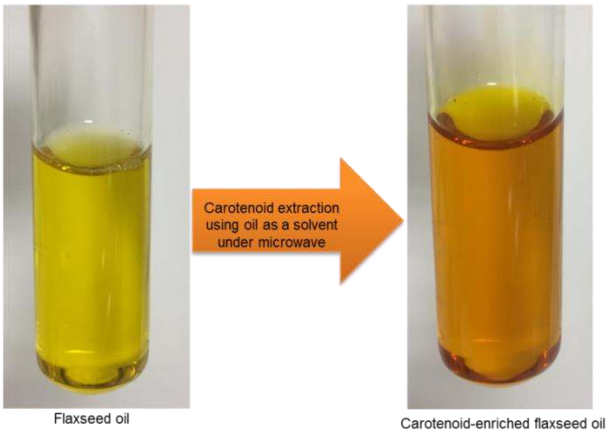


Figure 4.1 Original FO and the CEFO obtained in optimum conditions

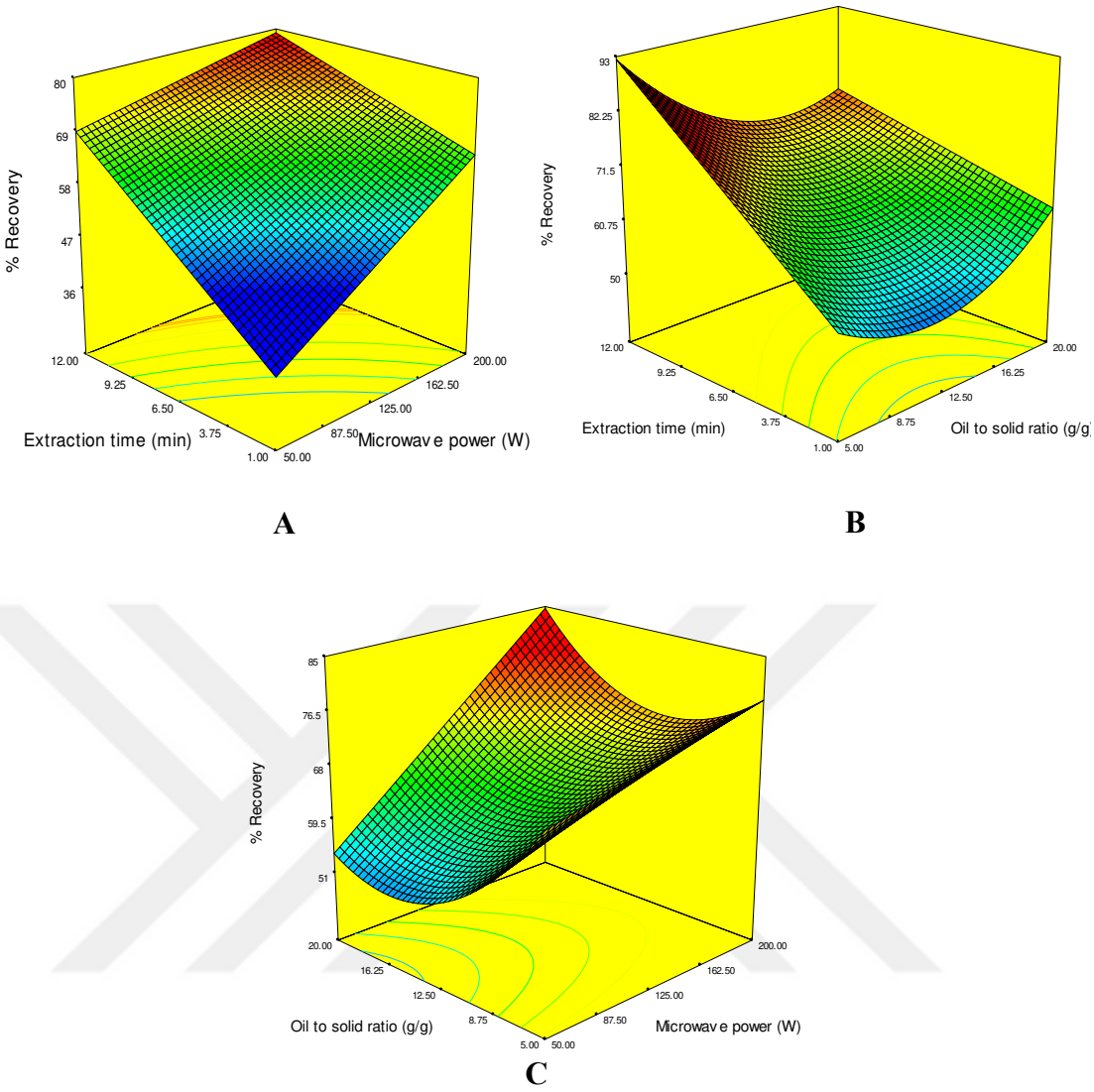


Figure 4.2 3D plots (A) extraction time (min) versus microwave power (W) (B) extraction time (min) versus oil to solid ratio (g/g) (C) oil to solid ratio (g/g) versus microwave power (W)

4.4 Comparison of MACE and CCE

The percent recovery of carotenoids was 77.48 % at optimum conditions (165W, 9.39 min and 8.06:1 g/g oil to solid ratio) using MACE. As it has been mentioned in introduction part, various conventional extraction conditions were applied to extract carotenoids into oil systems. It was important to compare the conventional extraction results with our finding to see how the recovery has been changed with time and to what extent. As seen in the Figure 4.3, the extraction yield for conventional extraction method increased with time in the first 30 min and almost 50 % of the carotenoids were extracted in this time period. The extraction yield of carotenoids

(about 87 %) reached to equilibrium at around 180 min and further increase in time did not affect the yield of carotenoid extraction up to 275 min. Percent recovery of carotenoid under microwave was calculated for extraction times at 1, 3, 5, 7 and 9.39 min on the basis of Eq. (4) while microwave power and oil to solid ratio were kept constant at optimal values. While about 78 % of total carotenoids were extracted within the first 9.39 min of microwave treatment, it was needed more than 120 min to achieve the same value for conventional treatment. Although conventional extraction method had a higher recovery (about 87 %) of total carotenoids from CPW, time required for extraction was quite long (around 180 min) resulting in consumption of considerable energy.

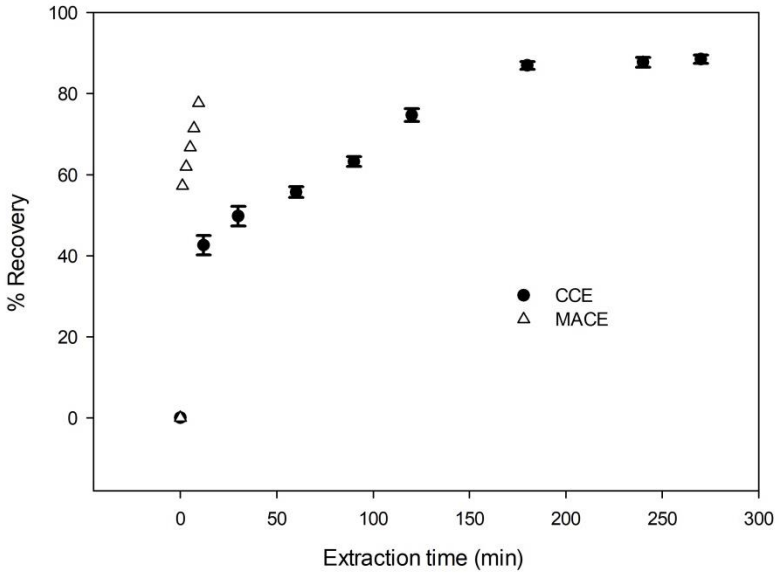


Figure 4.3 The effect of extraction time on recovery (%) of carotenoids (CCE: conventional carotenoid extraction; MACE: microwave-assisted carotenoid extraction)

4.5 Selected Physical and Chemical Properties of FO and CEFO

Quality parameters of CEFO obtained under the conditions of optimum microwave treatment were investigated and it was compared to those of FO. Physical and chemical quality parameters investigated for both oils were reported in Tables 4.3 and 4.4. It is shown that the fatty acids of FO were mainly constituted by linolenic (52.46 ± 0.02 %), oleic (19.77 ± 0.01 %), linoleic (15.35 ± 0.01 %), palmitic (6.25 ± 0.03 %), and stearic (4.41 ± 0.01 %) acids (Table 4.3). Although percentages of some fatty acids of CEFO were significantly (p<0.05) different from those of FO, fatty

acid values found for CEFO were still presenting a good quality FO. The similar findings have been reported in the previous study conducted by Yanik (2017).

Table 4.3 Fatty acid composition (% fatty acid), %unsaturated fatty acid, and ratio of omega 3: omega 6 fatty acids

Fatty acid	FO[1]	CEFO[2]
Palmitic acid (C16:0)	6.25 ± 0.03[a]	6.17 ± 0.05[a]
Palmitoleic acid (C16:1)	0.08 ± 0.02[a]	0.08 ± 0.00[a]
Stearic acid (C18:0)	4.41 ± 0.01[a]	4.55 ± 0.01[b]
Oleic acid (C18:1)	19.77 ± 0.01[b]	19.73 ± 0.01[a]
Linoleic acid (C18:2)	15.35 ± 0.01[b]	15.33 ± 0.01[a]
Linolenic acid (C18:3)	52.46 ± 0.02[a]	52.47 ± 0.03[a]
Eicosadienoic acid (C20:0)	0.22 ± 0.01[a]	0.21 ± 0.01[a]
Other	1.46 ± 0.02[a]	1.46 ± 0.04[a]
% Unsaturated fatty acids	87.67 ± 0.01[a]	87.61 ± 0.03[a]
Omega 3: omega 6 fatty acids	3.42 ± 0.00[a]	3.42 ± 0.01[a]

All the given values are means of three (n = 3) determinations ± SD
[ab]Means within a row with different letters are significantly different ($p < 0.05$)
[1]Flaxseed oil
[2]Carotenoid-enriched flaxseed oil

The Hunterlab L*, a* and b* parameters of FO and CEFO were compared. CEFO showed a higher a* and b* values as well as lower L* value (Table 4.4). This means that the CEFO was darker and more red and yellow in colour than FO. This is a clear indication of successful extraction of β-carotene into the FO during microwave treatment because β-carotene is known as the predominant phytochemical component in orange carrot and it is a deep orange-yellow in colour (Sun et al., 1996; Poudyal et al., 2010).

Acid, peroxide and p-anisidine values are important indicators of oil quality affected during processing and storage stages of oils. Peroxide and p-anisidine are the primary and secondary products of oil oxidation reactions, respectively; while acid value is a good indicator for hydrolytic reactions (J. W. Li et al., 2016). Acid values of FO and CEFO were found as 0.76 ± 0.08 and 1.16 ± 0.14 mg KOH/g oil, respectively (Table 4). Significant ($p<0.05$) increase in acid value was observed after the microwave treatment. This increase is probably due to the splitting of ester linkage of triglyceride molecules during microwave treatment (Hassanein et al., 2003). In a study conducted by Megahed (2011), acid value of FO exposed to microwave irradiation showed a similar trend. According to the Codex standard (Codex

Alimentarius, 2019), acid value of cold-pressed oil has to be less than 4.0 mg KOH/g oil. The results were lower than the limit established by Codex Alimentarius.

The peroxide values of FO and CEFO were determined as 0.79 ± 0.03 and 3.95 ± 0.09 milliequivalents (meq) peroxide/kg oil, respectively. Kreps et al. (2014) reported that the peroxide values of sunflower and corn oils grew significantly after a 10 min of microwave treatment. Even if the peroxide value showed a significant increase (p<0.05) during microwave treatment, the peroxide values of FO and CEFO were still well within the limit of up to 15 meq peroxide/ kg of oil (Codex Alimentarius, 2019).

P-anisidine values found for FO and CEFO were 0.018 ± 0.00 and 0.274 ± 0.00, respectively. A significant increase in p-anisidine value of CEFO was observed after microwave treatment. Chiavaro et al. (2010) reported that microwave treatment of canola oil, high oleic sunflower oil and peanut oil for 15 min led significant increase of p-anisidine value over fresh oils. However, p-anisidine value should be lower than 2 for good quality oil (Choo et al., 2007). The microwave-treated oil obtained in the present study is still below this value and it can be considered as a good quality oil after MACE.

Among the carotenoids, the β-carotene is the most abundant in the carrot processing waste (Sharma et al 2012). The percent β-carotene of the total carotenoid content was determined for enriched FO produced and CPW. Total carotenoid and β-carotene contents of CPW were found as 4132.83 ± 1.05 and 2507.97 ± 0.49 µg/g CPW powder, respectively. β-carotene constituted 60.7 % of the total carotenoid content in CPW. Total carotenoid and β-carotene contents of CEFO obtained under optimum conditions were 397.10 ± 0.8 and 236.35 ± 4.5 µg/g oil, respectively. It was determined that β-carotene accounted for 59.5% of carotenoids in CEFO. It is comprehended from the HPLC results that a large portion of carotenoid extracted from CPW was β-carotene (about 60%). β-carotene content of FO after MACE was increased significantly (p<0.001).

Table 4.4 Selected physical and chemical properties of FO and CEFO

Physical-chemical property	FO[1]	CEFO[2]
L^*	72.77 ± 0.08^b	58.69 ± 0.09^a
a^*	-2.53 ± 0.09^a	28.35 ± 0.1^b
b^*	77.01 ± 0.18^a	97.65 ± 0.21^b
Acid value (mg KOH/g oil)	0.76 ± 0.08^a	1.16 ± 0.14^b
Peroxide value (meq/kg oil)	0.79 ± 0.03^a	3.95 ± 0.09^b
p-anisidine value (unit)	0.018 ± 0.00^a	0.274 ± 0.00^b
β-carotene content (µg/g oil)	1.85 ± 0.42^a	236.35 ± 4.58^b
Total phenolic content (µg GAE/ g oil)	17.10 ± 0.99^a	214.05 ± 1.61^b
DPPH (%inhibition)	54.23 ± 1.37^a	70.67 ± 0.85^b

All the given values are means of three (n = 3) determinations ± SD
[ab]Means within a row with different letters are significantly different ($p < 0.05$)
[1]Flaxseed oil
[2]Carotenoid-enriched flaxseed oil

The results (Table 4.4) showed that TPC and antioxidant activity of FO increased after carotenoid extraction under microwave. TPC of CEFO (214.05 ± 1.61 µg GAE/g oil) was about twelve times higher than that of FO (17.10 ± 0.99 µg GAE/g oil). Furthermore, it was found that antioxidative properties of FO after carotenoid extraction were also improved. While DPPH scavenging activity of FO was 54.23 ± 1.37 %, this value reaches up 70.67 ± 0.85 % for CEFO. Presence of carotenoids such as β-carotene which were reported to have stronger antioxidant capacity (B. Hiranvarachat and Devahastin, 2014) in FO after the microwave extraction may account for the better results obtained for its antioxidant activity.

4.6 Emulsion Stability

Emulsion stability refers to the ability of emulsions to resist changes in their physicochemical properties over time (Y. T. Hu et al., 2017). The influences of individual factors on the stability of the emulsions were obtained by observing % separation of the emulsions after 24 h. There were 16 emulsions with various combinations of oil, pectin, maltodextrin and wax (Table 3.1). The stability study revealed that most of the emulsions were kinetically stable after 24 hours. The formation of a small separation layer was observed only in three emulsions. Percentage separation in these three emulsions for 12% CEFO 12:1, 15% CEFO 9:1 and 12:1 (ratios refer to MD/(LMP+SFW)) were determined as 2, 4 and 8%, respectively. As it is seen, at lower concentrations of CEFO (6 and 9%) there was no

phase separation. However, increased CEFO concentration resulted in phase separation at a limited level only in some of the emulsions.

Pectin is able to stabilize the emulsions by adsorbing onto the oil-droplet interface thus providing a physical barrier to coalescence (Leroux et al., 2003). However, pectin concentration is very important in terms of providing the emulsion stability as only a modest proportion of the pectin used as emulsifier adsorbs on the droplets (Akhtar et al., 2002). In the present study, one of the major factors which lead to phase separation of some emulsions might be an insufficient amount of pectin. Low quantity (less than 1 wt%) of added LMP is found to destabilize the emulsions, leading to the formation of oil droplets on the emulsions. On the other words, the higher amount of LMP in the emulsion diminished phase separation. This is mainly attributed to the specifically higher increase of continuous phase viscosity (Table 4.5). Similarly, a study investigating emulsion stabilizing properties of depolymerized pectin, oil in water emulsions containing 20% rapeseed oil showed excellent long-term stability at 4 wt% pectin concentration due to forming a relatively viscous emulsion. However, when concentration of pectin decreased from 4 to 1 wt%, it has been reported that there was very extensive serum separation (Akhtar et al., 2002).

Besides LMP, a lower concentration of SFW was observed to cause phase separation in the emulsions containing 12 and 15 % CEFO. SFW was completely melted as the emulsification process was carried out at 80 °C and therefore crystals cannot diffuse through the droplets causing coalescence. In the following cooling process, the wax crystallizes near the oil/water interface which prevents the droplet coalescence and stabilizes the droplets. So, the emulsion stability might also be enhanced with higher SFW concentration along with LMP. A study conducted by C. F. Li et al. (2009) has been reported that increasing wax concentration in the emulsion, particularly prepared at high temperature (85°C), significantly improved emulsion stability.

The emulsifying properties of pectin only may not always be sufficient to obtain stable emulsion (Maravic et al., 2019). Preliminary studies also showed that considerable phase separation was observed in all emulsions prepared with only pectin and wax (data not shown). Therefore, MD was included in emulsion system. MD was contributed to the stability of emulsions which contain 6 and 9 % CEFO. In

the emulsions containing 12 and 15 % CEFO, however, an increase in MD/(LMP+SFW) ratio (increase in concentration of MD) was not contributed further to the emulsion stabilization. This probably results from that the phase-separated emulsions had lower total water soluble solids (LMP and MD) and the higher concentration of MD was not able to provide the emulsion stabilization alone due to the lower LMP content in the emulsions. It has been reported in the literature that in emulsions containing maltodextrins as stabilizers, a sufficient amount emulsifying agent is required for the production of a stable emulsion because maltodextrins are not particularly surface-active (Klinkesorn et al., 2004).

4.7 Rheological Properties of Emulsion

Figures 4.4-4.7 show the rheograms of the flow behavior properties of emulsions prepared in different formulations. It can be seen that the shear stress of the emulsions increases with increasing shear rate. A power law model was used to explain the rheological behavior of the emulsions. The coefficients of determination (R^2) of the model were found to be very high (0.9926 to 0.9998). The K (consistency coefficient) and n (flow behavior index) values of the emulsions at different formulations were calculated by the power law model (Table 4.5). The K and n values of the emulsions obtained from 16 different runs are shown in Table 4.5. The K values obtained were between 7.7853–0.0872 and n values ranged between 0.7723 and 1.0933.

For all CEFO concentration, emulsion with 3:1 MD/(LMP+SFW) ratio had the smallest n value, which means that the degree of pseudoplasticity or shear thinning ability of emulsions was the highest. But when MD/(LMP+SFW) ratio increased, the n values of the emulsions were close to 1, indicating that the fluid behavior approaches Newtonian behavior. This may be attributed that the higher concentration of LMP led to a reduced collision frequency, reducing the mobility of oil droplets. These results are in agreement with those of other workers who have also observed an increase in pseudoplasticity with an increase in pectin concentration (Baumler et al., 2013; Muhammad et al., 2014). Moreover, pseudoplastic behavior of the emulsions may have promoted by the higher concentration of SFW (along with LMP) due to exhibiting a typical shear-thinning flow of the emulsions which contain wax (C. F. Li et al., 2009). However, for a constant CEFO level, the increase in MD

ratio led to emulsions to approach Newtonian flow behavior. This was not an unexpected result, since the study conducted by Udomrati et al. (2013) reported that the emulsions which contain maltodextrin at different concentrations (5-35 %) exhibited Newtonian flow behavior.

Apparent viscosities of emulsions at a specified shear rate (50 s^{-1}) were in the range of 0.0869-3.1939 Pa.s. Lower emulsion viscosities were observed with the increase in CEFO concentration. This could be explained with the lower amount of LMP present in the emulsions formed with higher CEFO concentration, for the same total solid content, resulting in a significantly less pronounced thickening effect and thus in less viscous emulsions. Tonon et al. (2011) also verified a decrease of viscosity with the increase in the mass ratio of oil for the same total solid content.

The results also showed that the increase in LMP + SFW concentration in the emulsions containing 6 % CEFO led to a significant increase in apparent viscosity. Approximately a three-fold rise in pectin concentration (1.23 to 4 g) caused about an eight-fold increase in the apparent viscosity of the emulsions (0.3769 to 3.1939 Pa.s). The emulsions that contained 9, 12 and 15% CEFO also showed similar behavior. This is probably due to the fact that increasing the content of LMP increases the viscosity of the emulsion. Similarly, Baumler et al. (2013); Chalapud et al. (2018) have been reported that an increase in pectin concentration resulted in a significant increase in apparent viscosity. In addition to LMP, an increase in SFW concentration might have led to more pronounced increase in viscosity. As mentioned above, wax starts to crystallize near the oil/water interface during the cooling process following the emulsification. The crystallization of wax may increase emulsion viscosity. These results are in agreement with those of other workers who have also observed an increase in the emulsion viscosity when wax concentration increases (C. F. Li et al., 2009). On the contrary, the increase in MD ratio in the emulsions at a constant CEFO level was observed to cause a decrease in apparent viscosity. This decrease in viscosity is basically because of a lower content of LMP+SFW with increasing ratio of MD in the emulsions. Even if the total soluble solid is constant in the emulsion, the decrease in the amounts of emulsion stabilizing components, LMP and SFW, might be the main reason for this decrease in the viscosity. So, the water binding and

the gel formation abilities of pectin and crystallization effect of wax are the main contributors to the viscosity of the emulsion.

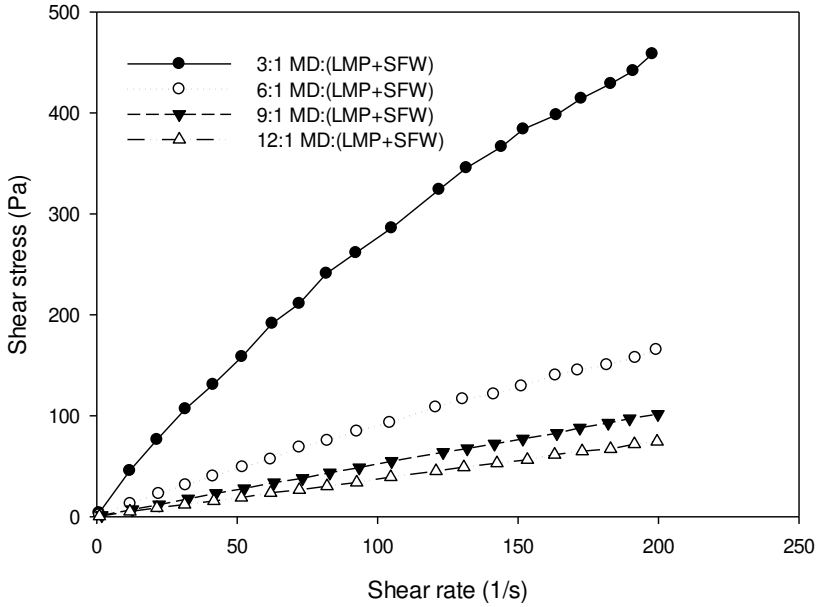


Figure 4.4 Flow behavior rheograms of emulsions contained 6 % oil concentration at constant temperature (MD: maltodextrin; LMP: low methoxylated pectin; SFW: sunflower wax)

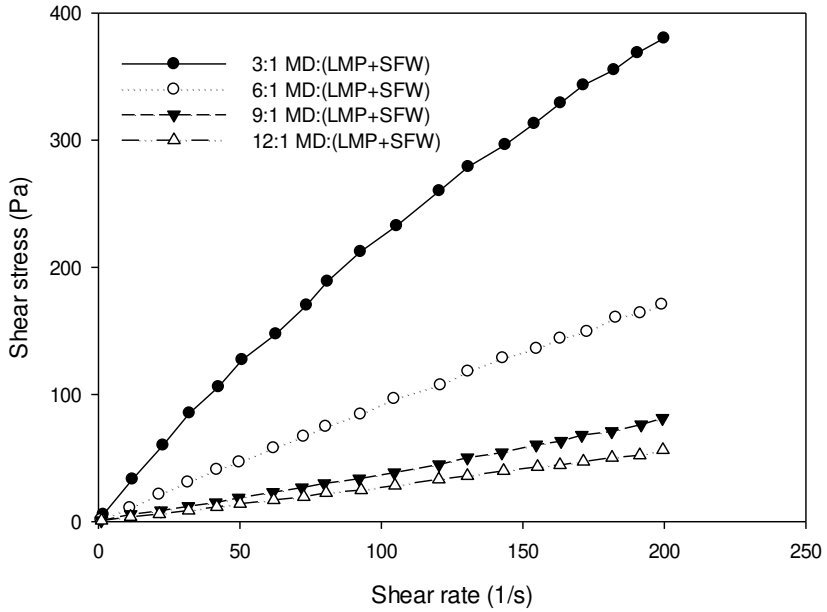


Figure 4.5 Flow behavior rheograms of emulsions contained 9 % oil concentration at constant temperature (MD: maltodextrin; LMP: low methoxylated pectin; SFW: sunflower wax)

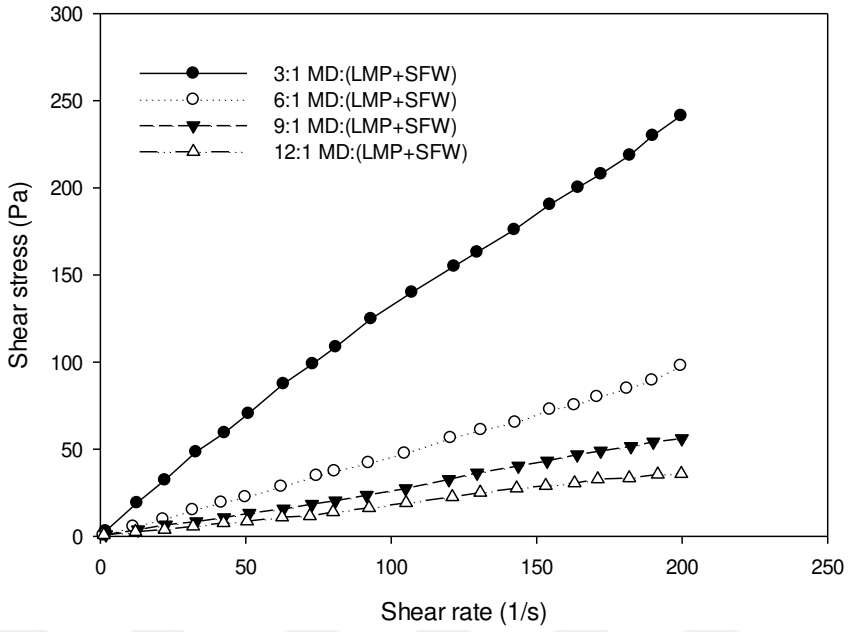


Figure 4.6 Flow behavior rheograms of emulsions contained 12 % oil concentration at constant temperature (MD: maltodextrin; LMP: low methoxylated pectin; SFW: sunflower wax)

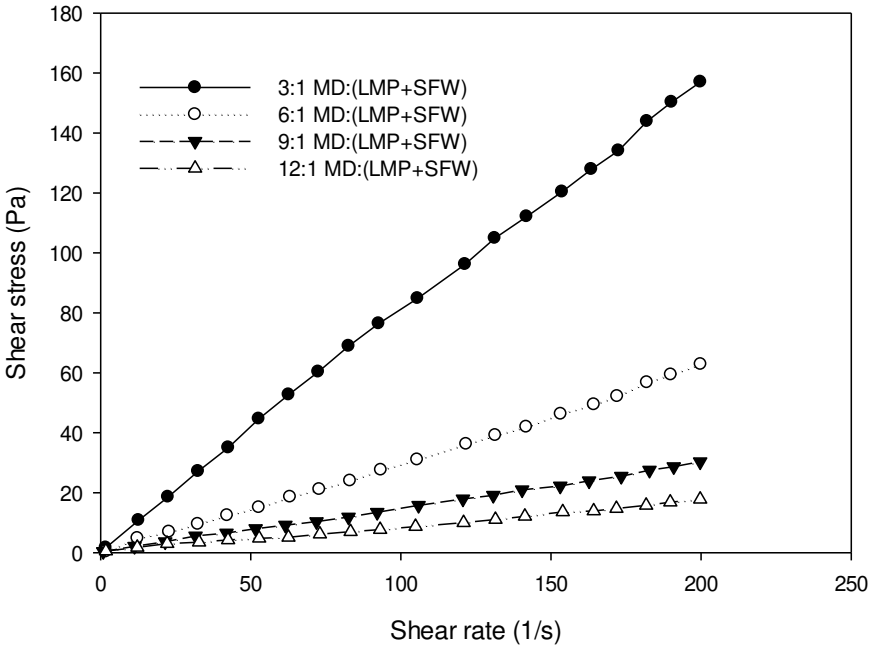


Figure 4.7 Flow behavior rheograms of emulsions contained 15 % oil concentration at constant temperature (MD: maltodextrin; LMP: low methoxylated pectin; SFW: sunflower wax)

Table 4.5 Flow behavior (rheological) properties and apparent viscosity values at 50 s^{-1}, temperature = 20 ^{o}C

CEFO concentration in emulsion (%)	MD / (LMP+SFW) (g/g)	Power law model			Apparent viscosity (Pa.s)
		K ($Pa.s^n$)	n	R^2	
6	3:1	7.7853 ± 0.0858	0.7723 ± 0.0025	0.9984	3.1939 ± 0.0043[a]
	6:1	1.4971 ± 0.0312	0.8892 ± 0.0046	0.9992	0.9702 ± 0.0028[b]
	9:1	0.6310 ± 0.0254	0.9582 ± 0.0084	0.9998	0.5355± 0.0039[c]
	12:1	0.3869 ± 0.0079	0.9933 ± 0.0031	0.9995	0.3769 ± 0.0031[d]
9	3:1	5.1902 ± 0.0054	0.8137 ± 0.0002	0.9990	2.5037 ± 0.0005[a]
	6:1	1.2767 ± 0.0208	0.9238 ± 0.0004	0.9995	0.9476 ± 0.0170[b]
	9:1	0.2854 ± 0.0022	1.0617 ± 0.0019	0.9993	0.3632 ± 0.0001[c]
	12:1	0.2711 ± 0.0108	1.0023 ± 0.0103	0.9995	0.2733 ± 0.0001[d]
12	3:1	2.2092 ± 0.0069	0.8851 ± 0.0001	0.9996	1.4093 ± 0.0052[a]
	6:1	0.3936 ± 0.0082	1.0353 ± 0.0039	0.9988	0.4518 ± 0.0025[b]
	9:1	0.1740 ± 0.0042	1.0933 ± 0.0039	0.9986	0.2505 ± 0.0022[c]
	12:1	0.1471 ± 0.0139	1.0470 ± 0.0198	0.9963	0.1764 ± 0.0030[d]
15	3:1	1.0113 ± 0.0004	0.9511 ± 0.0003	0.9996	0.8352 ± 0.0013[a]
	6:1	0.2178 ± 0.0059	1.0659 ± 0.0045	0.9988	0.2817 ± 0.0027[b]
	9:1	0.1476 ± 0.0036	1.0016 ± 0.0048	0.9983	0.1485 ± 0.0008[c]
	12:1	0.0872 ± 0.0043	0.9995 ± 0.0107	0.9926	0.0869 ± 0.0007[d]

All the given values are means of three (n = 3) determinations ± SD.
[a,b,c,d] Means within a row with different letters for each oil concentration are significantly different ($p < 0.05$).
MD: maltodextrin; LMP: low methoxylated pectin; SFW: sunflower wax

4.8 Emulsion Particle (Droplet) Size Analysis

The droplet size and droplet size distribution of the dispersed phase in the emulsion are important parameters which are directly related to the emulsification process and provide information on emulsion stability. Generally, small droplet size and narrow and monomodal droplet size distribution indicate that the emulsion is more stable (Guo et al., 2014). The droplet size distribution of the emulsions was determined to evaluate the extent of emulsion droplet flocculation. It can be seen from Figures 4.8-4.11, there is no considerable visible difference between the droplet size distribution of emulsions prepared with different CEFO concentration and MD/(LMP+SFW) proportions. All emulsions showed very narrow and uniform droplet size distributions. Results indicated that there was no extensive aggregation between the oil droplets for all emulsions.

The average particle size values ($D_{4,3}$) of the emulsions prepared at different concentrations are given in Table 4.6. According to the results, the average particle sizes of the emulsions varied in the range of 5.85 and 11.35 μm. At low CEFO concentrations (6 and 9%), it was observed that the average particle size increased with increasing MD/(LMP + SFW) (g/g) ratio. In other words, the increase in LMP + SFW concentration in emulsions with CEFO contents of 6% and 9% resulted in a reduction in the average particle size. This is probably attributed that the pectin enhances droplet stabilizing mechanism at its higher concentration. This increase in average particle size, when MD/(LMP + SFW) (g/g) ratio increases, is not significant at 6% CEFO concentration, but is more pronounced in emulsions containing 9% CEFO. It has been reported in previous studies that the average particle size decreases with increasing the pectin concentration (Akhtar et al., 2002; Chalapud et al., 2018). However, if CEFO concentration in the emulsions was 12% or more, the increase in MD/(LMP + SFW) (g/g) ratio led to a decrease in the average particle size. Pectin and maltodextrin are water trapping components. When the oil concentration increases, the amount of water soluble solids (pectin and maltodextrin) decreases (from 23.38 to 13.75%). The decrease in water trapping components and the increase in non-polar component concentration (SFW + CEFO) might have caused the oil droplets to form large particles more readily. Therefore, at higher CEFO concentrations (12% and 15%), the increase in the total amount of solids

(LMP + MD) rather than the effect of pectin alone leads to the formation of more stable emulsions, decreasing the average particle sizes.

Table 4.6 Average particle size of emulsions prepared in different formulations

CEFO concentration in emulsion (%)	MD / (LMP+SFW) (g/g)	$D_{[4.3]}$ (μm)
6	3:1	5.85 ± 0.02^{a}
	6:1	6.07 ± 0.13^{ab}
	9:1	6.11 ± 0.17^{ab}
	12:1	6.32 ± 0.12^{b}
9	3:1	6.74 ± 0.04^{a}
	6:1	7.62 ± 0.08^{b}
	9:1	9.32 ± 0.22^{c}
	12:1	9.21 ± 0.15^{c}
12	3:1	9.19 ± 0.18^{b}
	6:1	9.27 ± 0.03^{b}
	9:1	7.41 ± 0.13^{a}
	12:1	7.42 ± 0.37^{a}
15	3:1	11.35 ± 0.26^{b}
	6:1	9.01 ± 0.17^{a}
	9:1	9.41 ± 0.31^{a}
	12:1	9.39 ± 0.32^{a}

All the given values are means of three (n = 3) determinations ± SD.
[a,b,c,] Means within a row with different letters for each oil concentration are significantly different ($p < 0.05$). (MD: maltodextrin; LMP: low methoxylated pectin; SFW: sunflower wax)

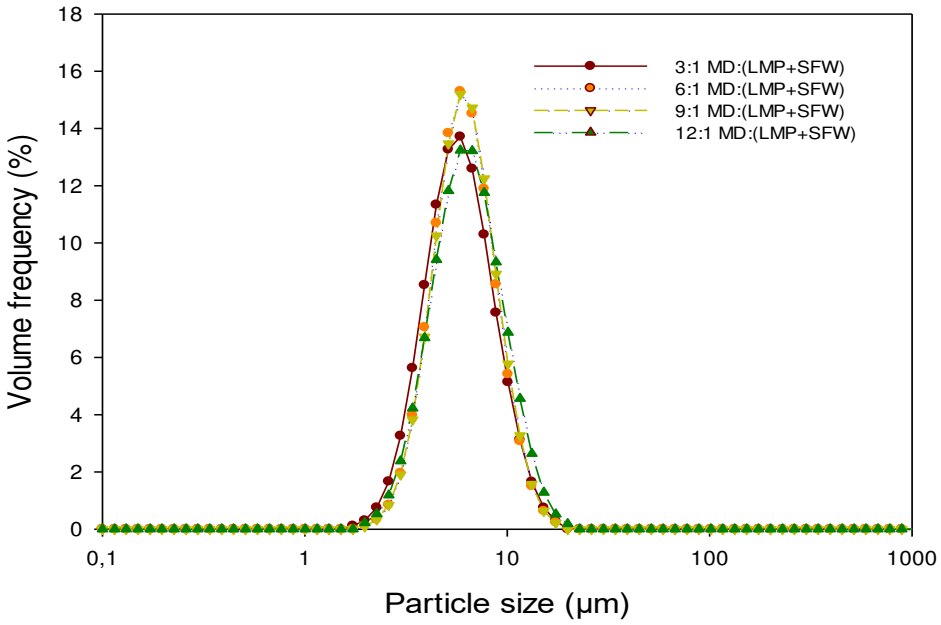


Figure 4.8 Particle size distributions of the emulsions contained 6 % oil concentration (MD: maltodextrin; LMP: low methoxylated pectin; SFW: sunflower wax)

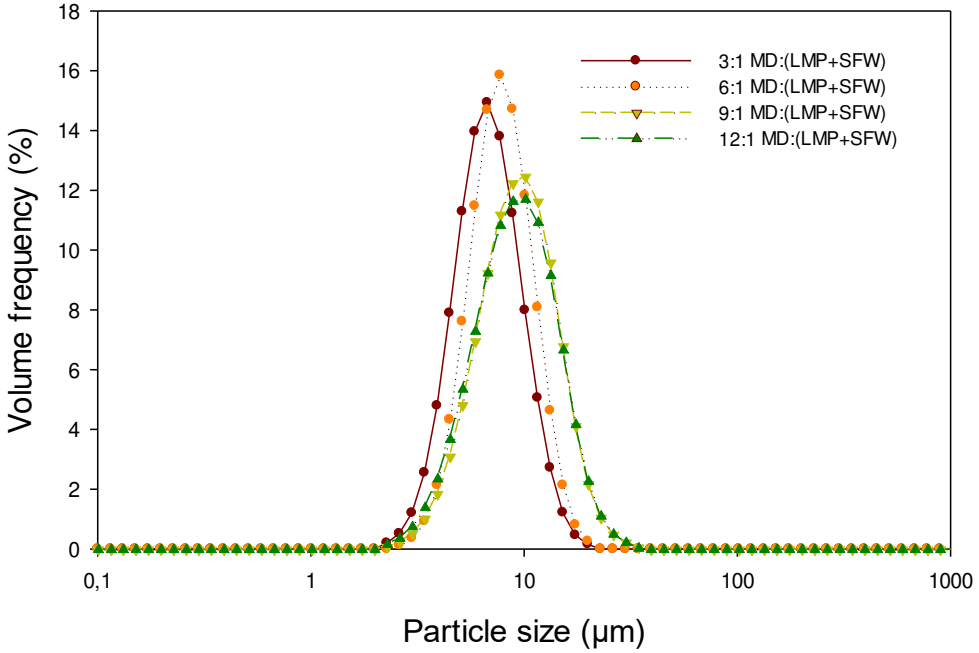


Figure 4.9 Particle size distributions of the emulsions contained 9% oil concentration (MD: maltodextrin; LMP: low methoxylated pectin; SFW: sunflower wax)

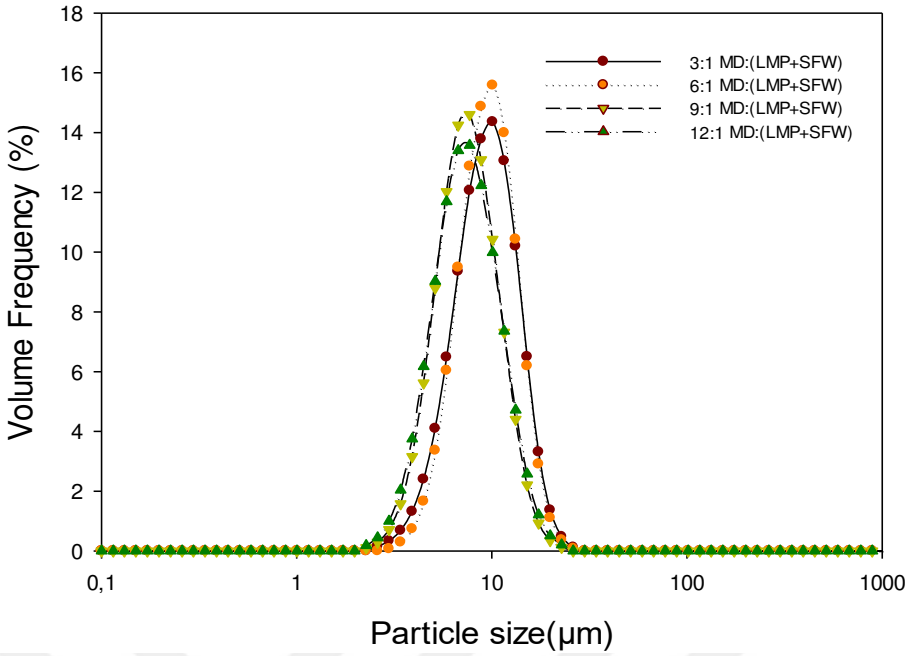


Figure 4.10 Particle size distributions of the emulsions contained 12 % oil concentration (MD: maltodextrin; LMP: low methoxylated pectin; SFW: sunflower wax)

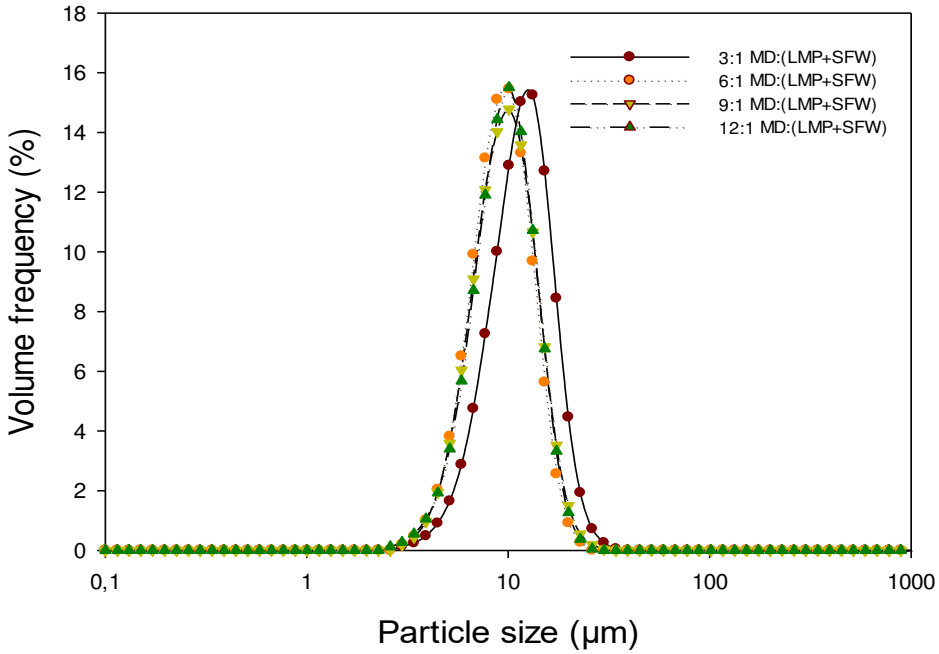


Figure 4.11 Particle size distributions of the emulsions contained 15 % oil concentration (MD: maltodextrin; LMP: low methoxylated pectin; SFW: sunflower wax)

4.9 Encapsulation Efficiency

Table 4.7 shows the encapsulation efficiency of the powders produced by SFD. The encapsulation efficiency values varied from 43.5 to 70.9 %. Emulsions which contain 6 and 9% CEFO concentration and 3:1 ratio of MD/(LMP+SFW) were not able to atomize due to their high viscosities (3.1939 Pa.s for 6 % CEFO and 2.5037 Pa.s for 9% CEFO at 50 s^{-1}) and so encapsulation efficiency values for those emulsions are not available to be reported. It was observed that the encapsulation efficiency was significantly influenced by MD/(LMP+SFW) ratio and oil concentration. The increase in oil concentration led to lower encapsulation efficiency. It can be attributed to lower amount of pectin which became insufficient for covering the oil droplets fully. This could be resulted in higher amount of surface oil which was not encapsulated. The study of Karthik and Anandharamakrishnan (2013) is the only report on the encapsulation of oils by SFD technique. However, they did not examine the effect of the oil concentration on the encapsulation efficiency. Nevertheless, there are findings in the literature that an increase in oil concentration in the emulsion generally reduces encapsulation efficiency. In a study by Tonon et al. (2011), FO was encapsulated by SD technique using gum arabic as the wall material. They reported that the encapsulation efficiency decreased when FO concentration increased similar to our findings.

MD/(LMP+SFW) ratio had also an important impact on the encapsulation efficiency. The results showed that the increase in MD/(LMP+SFW) ratio in the emulsions at a constant CEFO level led to a significant decrease in the encapsulation efficiency. In other words, the increase in LMP+SFW ratio resulted in higher encapsulation efficiency. The increase in encapsulation efficiency with increasing LMP+SFW concentration in emulsion at a constant CEFO level might be due to higher viscosity of emulsion. Pectin has a rapid gel-forming ability and makes emulsion more viscous (Attallah et al., 2020). Higher viscosity leads to the reduction of the emulsion components' movements inside the droplets. This makes the oil migration difficult through the particle surface. Thus, the increase in pectin concentration in the emulsion might cause the powder to be produced with lower surface oil.

Table 4.7 Encapsulation efficiency of the powders produced by spray freeze drying

CEFO[1] concentration (%)	MD/ (LMP+SFW)[2] (g/g)	Efficiency (%)
6	3:1	-
	6:1	70.9±0.8[a]
	9:1	65.5±0.3[b]
	12:1	54.4±1.6[c]
9	3:1	-
	6:1	60.5±0.1[a]
	9:1	55.6±0.9[a]
	12:1	47.8±1.7[b]
12	3:1	64.4±1.3[a]
	6:1	56.1±0.5[b]
	9:1	53.4±1.9[b]
	12:1	45.1±0.6[c]
15	3:1	62.7±1.5[a]
	6:1	56.7±0.3[b]
	9:1	53.7±0.5[c]
	12:1	43.5±0.2[d]

All the given values are means of three (n = 3) determinations ± SD
Means within a row with different letters for each oil concentration are significantly different ($p < 0.05$) (CEFO: Carotenoid-enriched flaxseed oil; MD: maltodextrin; LMP: low methoxylated pectin; SFW: sunflower wax)

In oil encapsulation, SD is the most widely used encapsulation technique. The best emulsion formulation which resulted in the highest encapsulation efficiency (6% CEFO 6:1 MD/(LMP+SFW) ratio) by SFD technique was used to produce the powder by SD, as well. As seen in Figure 4.12, the encapsulation efficiency (oil retention) was higher (yielded 89.8%) in SD technique as compared with SFD (yielded 70.9 %) technique. This clearly depicts the superiority of SD technique as it covers the core material very well. Lower encapsulation efficiency by SFD technique could be explained by the tendency of the particles to agglomerate owing to higher surface energy in the spray freezing stage (Anandharamakrishnan and Ishwarya, 2015). Similarly, in the encapsulation of docosahexaenoic acid (DHA) by three different techniques (SFD, SD and freeze-drying), Karthik and Anandharamakrishnan (2013) reported that the encapsulation efficiency of the powder product obtained by SD was higher than that obtained by SFD.

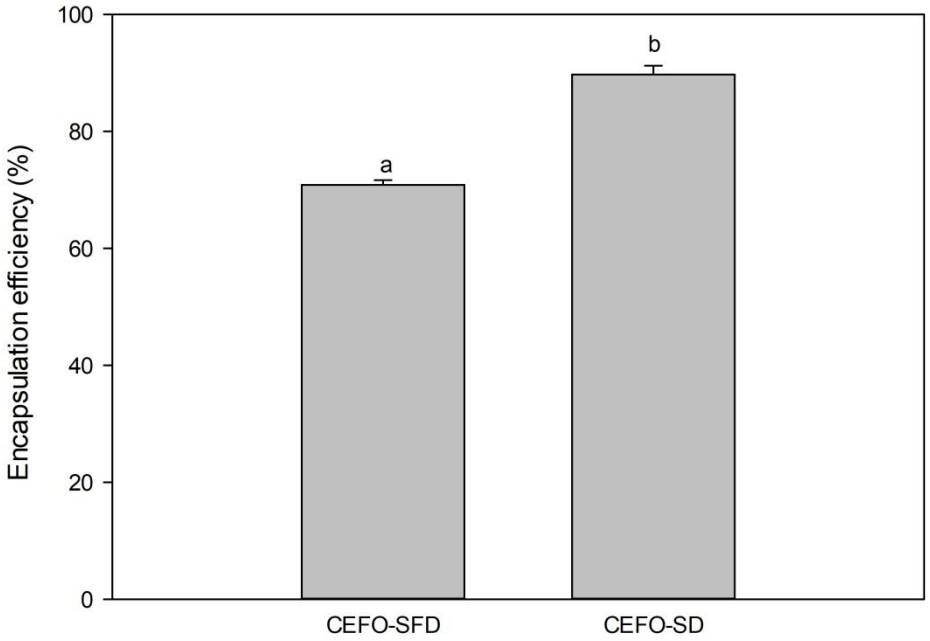


Figure 4.12 Encapsulation efficiency (%) of CEFO encapsules obtained by SFD and SD methods (CEFO: carotenoid enriched flaxseed oil; SFD: spray freeze drying; SD: spray drying)

4.10 Powder Properties

Moisture content plays an important role in determining the shelf life of the encapsulated powder products. High moisture content causes some problems affecting the physical and chemical stability and acceptability of products such as mold growth and agglomeration in the powder products. However, low moisture content which refers basically low water activity is preferable for obtaining powder with better characteristics such as prevention of oil oxidation. In general, a maximum moisture content of 3-4% is allowed for most powders used in the food industry (Gallardo et al., 2013). In this study, the moisture contents of the encapsulated powders obtained by SFD and SD techniques were 1.13±0.04 and 2.41±0.24%, respectively (Table 4.8). The moisture contents of the powders obtained by two different techniques are quite acceptable in terms of maintaining physical and chemical stability. According to the results, the encapsulation technique had statistically significant effect on the moisture content of the CEFO capsules (p<0.05). The moisture content of the powders encapsulated by SFD technique is lower compared to SD. Even if their moisture contents are quite close to each other, a higher moisture content of SD powder can be explained by the selection of relatively low air temperature (135^{o}C) and its short contact time with hot air. Parthasarathi and

Anandharamakrishnan (2016) conducted a study in which vitamin E has been encapsulated by using three different techniques (SFD, SD and freeze-drying). They found that SFD technique resulted in the formation of encapsulated powder with the lowest moisture content similar to our findings.

Table 4.8 Selected physical properties of the powders produced by spray freeze drying and spray drying

Properties	CEFO-SFD[1]	CEFO-SD[2]
Moisture content (%)	1.13±0.04[a]	2.41±0.24[b]
Practical size (D_{43} (μm))	38.97±0.70[b]	12.95±0.02[a]
Flowing properties		
Bulk density (kg/m^3)	192.50±0.5[a]	240.85±2.05[b]
Hausner's ratio	1.32±0.01[a]	1.68±0.02[b]
Carr's index	24.51±0.34[a]	40.62±0.81[b]

All the given values are means of three (n = 3) determinations ± SD
Means within a row with different letters are significantly different ($p < 0.05$)
[1]Carotenoid-enriched flaxseed oil-spray freeze drying
[2]Carotenoid-enriched flaxseed oil-spray drying

The average particle sizes of powders encapsulated by two different techniques are given in Table 4.8. While the average particle size of the capsules obtained by SFD was 38.97 μm, the average particle size of the capsules obtained by SD was 12.95 μm. The larger particle size of the capsules obtained by SFD might be due to the agglomerated ice crystals during the spray freezing step of the SFD. Hundre et al. (2015) used SFD, SD and freeze drying techniques in encapsulation of vanillin. They reported that the particle sizes of capsules obtained by SFD were much larger compared to that of SD, depending on the wall material.

Figure 4.13 shows the particle size distributions of powders encapsulated by two different techniques. A monomodal distribution was observed for encapsulated powder by SD, meaning a homogeneous particle size distribution. However, the particle size distribution of the powder encapsulated by SFD presented a bimodal behavior which shows that the particle size distribution is not homogeneous like in the case of that of SD powder. This can be attributed to agglomeration of some of small particles during the freezing step of SFD as mentioned above.

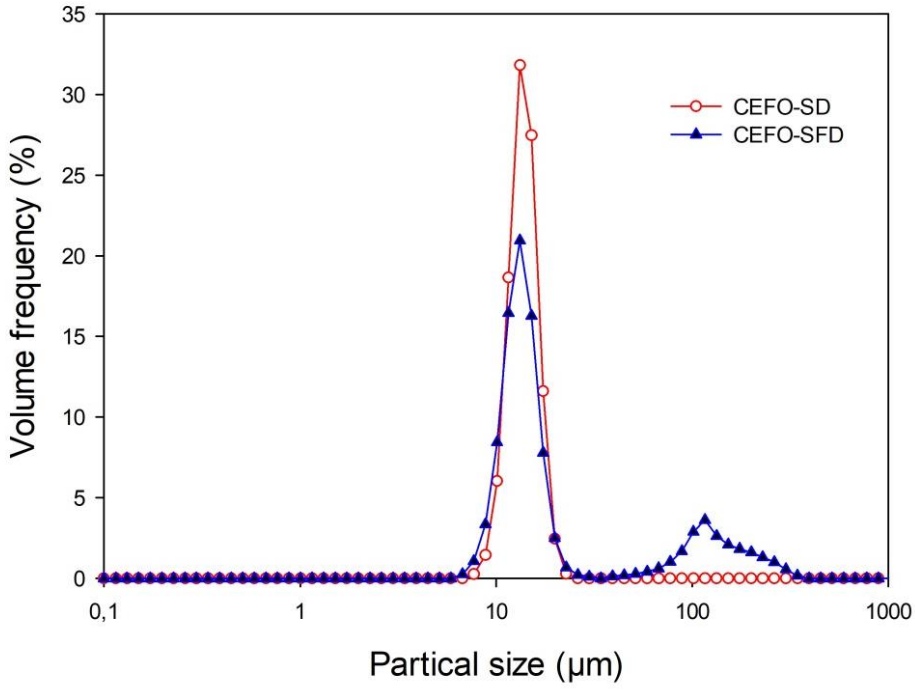


Figure 4.13 Particle size distributions of CEFO encapsules obtained by SFD and SD methods (CEFO: carotenoid enriched flaxseed oil; SFD: spray freeze drying; SD: spray drying)

Physical parameters such as bulk density and tapped density affect the flowing properties and storage stability of the powder. The increase in bulk density reduces the amount of trapped air in the spaces between the particles. This results in reduction of oxidation risk of the powder product during storage and dependently increasing storage stability (Carneiro et al., 2013). As it is known, powder products with high bulk density require less storage space as they reduce package volume compared to powders with low bulk density. In this study, the bulk densities of powders obtained by using SFD and SD techniques were determined to be 192.50 and 240.85 kg/m^3, respectively (Table 4.8). The results showed that the encapsulation technique had a significant effect on bulk density. A lower bulk density of the powders encapsulated by the SFD technique might be due to the larger particle size compared to that of SD. These results are in agreement with those of other researchers who have also observed a lower bulk density for SFD compared to that of SD (Brunaugh et al., 2019).

The flowing characteristics of the CEFO powder encapsulated by two different techniques were determined by evaluating Carr's index (CI) and Hausner's ratio (HR). CI and HR values of CEFO-SFD powder were 24.51±0.34 and 1.32±0.01,

respectively. On the other hand, CI and HR values of CEFO-SD powder were 40.62±0.81 and 1.68±0.02, respectively. Flow characteristic of CEFO-SFD powder was considered as "possible" by its CI (21-25) and HR (1.26–1.34) (Shah et al., 2008). However, flow characteristic of CEFO-SD powder was considered as "very very poor" flow type materials by its CI (>38) and HR (>1.60) (Shah et al., 2008). Lower CI or lower HR of a material indicates better flow properties than higher ones (Shah et al., 2008). According to the results obtained in the study, the flowability of the encapsulated powder product obtained by SFD was much better than that obtained by SD. When the relationship of CI and HO values with particle size of a powder was considered, the particle size is an important factor in the determination of flowability characteristics. Smaller particle size increases cohesion and so flowability of the powder decreases significantly (Fitzpatrick et al., 2007). This might be the reason of lower flowability of the powder obtained by SD technique.

4.11 Powder Morphology

The morphology of the CEFO capsules encapsulated with two techniques, namely SFD and SD, has been examined by using SEM. The SEM images obtained are shown in Figure 4.14. For CEFO-SD powder, wrinkled surface with dents of the capsules was observed, but there were no cracks and pores. The wrinkled structures have been explained by the high inlet drying air temperature leading to a rapid withdrawal of surface water (Rajabi et al., 2015). The main factor causing wrinkle is the large temperature difference between drying air and powder particles. SD particles had irregular spherical and inwardly buckled shapes. This could be because of initial expansion due to the increased air volume in particle, then shrinkage in the colder parts of the dryer. A compressive stress on the particles due to capillary forces and the rapid removal of moisture could be another reason of this morphology (Foerster et al., 2016). It has been reported that inwardly buckled shape is less at higher drying temperatures (200^{o}C) (more spherical particles), however, wrinkling and inwardly buckled in shape are more pronounced at low drying temperatures (120^{o}C) (Nijdam and Langrish, 2005). In this study, SD was carried out at 130^{o}C in order to keep oxidation minimum while providing a good encapsulation of CEFO. Hence, it can be said that inwardly buckled shape formed on the capsule surface due to the relatively low drying temperature.

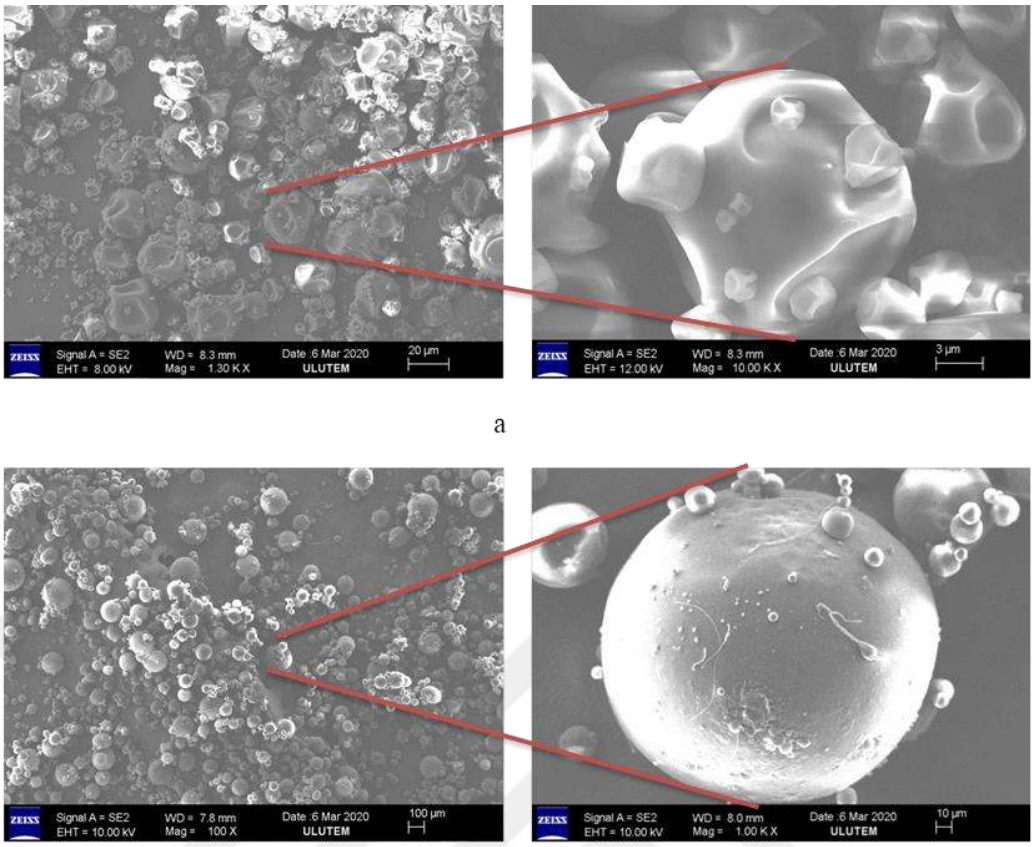


Figure 4.14 CEFO capsules obtained by two different encapsulation techniques (a) SEM image of CEFO-SD; (b) SEM image of CEFO- SFD (CEFO: carotenoid enriched flaxseed oil; SFD: spray freeze drying; SD: spray drying)

The morphology of the particles obtained by SFD process depends on the applied steps (spray freezing and freeze drying). In SFD process, the most important factor affecting morphology is freezing and nucleation rates (Wanning et al., 2015). In case of a significant temperature gradient in the droplet due to rapid cooling, the first nucleus formation begins on the surface of the droplet. If there is an even temperature distribution, homogeneous nucleation may also occur inside the droplet. Surface nucleation causes characteristic patterns to form in the pores of the dried particles (İşleroğlu et al., 2018). If the freezing phase is carried out properly, there is no change in the microstructure except the formation of pores due to the removal of water in particles. Hence, the spherical shape and size of the particles are preserved (Anandharamakrishnan and Ishwarya, 2015; Rajam and Anandharamakrishnan, 2015). When the SEM images of SFD powders were examined, it was observed that

the particles had perfect spherical shape and there were no many pores on their surface (a relatively smooth surface). Moreover, agglomeration of same particles was observed as shown in Figure 4.14b. This confirms the bimodal behavior in the particle size distribution.

FO and CEFO capsules obtained by two different encapsulation techniques are shown in Figure 4.15.

Figure 4.15 Images of FO and CEFO capsules obtained by two different encapsulation techniques (FO: flaxseed oil; CEFO: carotenoid-enriched flaxseed oil; SFD: spray freeze drying; SD: spray drying)

SFD particles generally have many pores on the surface due to water sublimation during freeze drying (Ishwarya et al., 2015). In the present study, however, the particles did not show a very porous structure. The limited number of pores in the structure might be attributed to the filling of the pores with wax which has been frozen on the surface of the particles. Another explanation might be the tendency of SFD capsules to form a skin covering the porous microstructure below (Ishwarya et al., 2015; Rajam and Anandharamakrishnan, 2015). The formation of a less porous structure can enhance the mechanical resistance of the particles during transport, as

well as reduce the risk of oxidation of the particles (Ali and Lamprecht, 2014; Rajam and Anandharamakrishnan, 2015).

4.12 Oxidative Stability

FO was enriched with carotenoids using microwave technology before the encapsulation process. As it is known, carotenoids, especially β-carotene, show high antioxidant properties (Tribble, 1999). In that respect, carotenoid enriched emulsions were used in the production of encapsulated powders to observe the effect of carotenoids (236.35 μg β-carotene/g oil) on oxidative stability during two encapsulation processes (SFD and SD) and storage. In order to observe this effect, FO and CEFO were encapsulated using SFD and SD techniques.

Table 4.9 shows PV and TBARS values of FO and CEFO before and after the encapsulation techniques (SFD and SD). PV is a measure of the oxidation level and increases with increasing drying temperature because the high air temperature provides more accessible energy, which accelerates oil oxidation (Tonon et al., 2011). When the effect of encapsulation techniques on PV for both FO and CEFO was examined, it was seen that SFD process had no significant effect on PV (p>0.05). This might be due to the fact that SFD process was carried out at low temperature and under vacuum (in the spray freezing stage at -195^{o}C and in the freeze drying stage under vacuum at -45^{o}C). However, PV of FO capsules, which was encapsulated by SD process, increased to 6.39 meq O_2/kg oil (p<0.05). The increase in PV is related to the high temperature (135^{o}C) used in SD. According to the researchers using SD for encapsulation, the rapid formation of the particle crust during the SD process creates a resistance to the evaporation of trapped water in the particle, as a result of which a rapid temperature increase occurs in the particle and prolongs the high temperature exposure of PUFAs that can easily oxidize (Melgosa et al., 2019). As a result, the oxidation level and PV of powder increase after SD. However, a remarkable result is that it was no observed increase in PV of CEFO capsule produced by SD. As seen in Table 4.9, the initial PV of CEFO is higher than that of FO. This is because FO was subjected to microwave radiation during the carotenoid enrichment process. Although CEFO had a higher initial PV, carotenoids protected the oil quite well from oxidation during encapsulation by SD. This is due to

the fact that carotenoids with antioxidant properties inactivate free radicals such as lipid hydroperoxide, which may be formed during SD process.

Table 4.9 Oxidation products of the encapsulated oil produced by spray freeze drying and spray drying

	PV (meq O_2/kg oil)	TBARS (mmol/kg oil)
FO	0.79 ± 0.03^{a}	0.11 ± 0.02^{a}
FO-SFD	0.81 ± 0.07^{a}	0.14 ± 0.03^{a}
FO-SD	6.39 ± 0.11^{b}	0.40 ± 0.01^{b}
CEFO	3.95 ± 0.09^{A}	0.17 ± 0.01^{A}
CEFO-SFD	3.96 ± 0.01^{A}	0.18 ± 0.03^{A}
CEFO-SD	3.97 ± 0.03^{A}	0.23 ± 0.03^{A}

All the given values are means of three (n = 3) determinations ± SD
Means within a row with different letters are significantly different ($p< 0.05$)
FO: flaxseed oil; CEFO: carotenoid-enriched flaxseed oil; SFD: spray freeze drying; SD: spray drying

PV and TBARS of FO and CEFO capsules obtained using two different encapsulation techniques were monitored for 45 days at 25°C (Figures 4.16 and 4.17). While PV of FO-SFD powder increased to 3.25 ± 0.21 meq O_2/kg oil at the end of the storage period, PV of FO-SD powder reached up to 50.18 ± 0.42 meq O_2/kg oil. Having such a high oxidation rate in FO-SD powder may be related to high initial PV (6.39 ± 0.11 meq O_2/kg oil), passing the induction period of oxidation rapidly. In a similar study conducted by Melgosa et al. (2019), omega-3 fatty acid was encapsulated by SD. They found that PV of the omega-3 fatty acid powder was 28.0 ± 1.6 meq O_2/kg oil after encapsulation process and this value increased to 66.0 ± 0.4 meq O_2/kg oil at the end of storage for 28 days at 4°C. Despite a lower storage temperature and shorter storage time than what it has been used in this study, they found a much higher PV results than our findings. This remarkable difference might be related to higher initial PV of the omega-3 fatty acid powder obtained in their study.

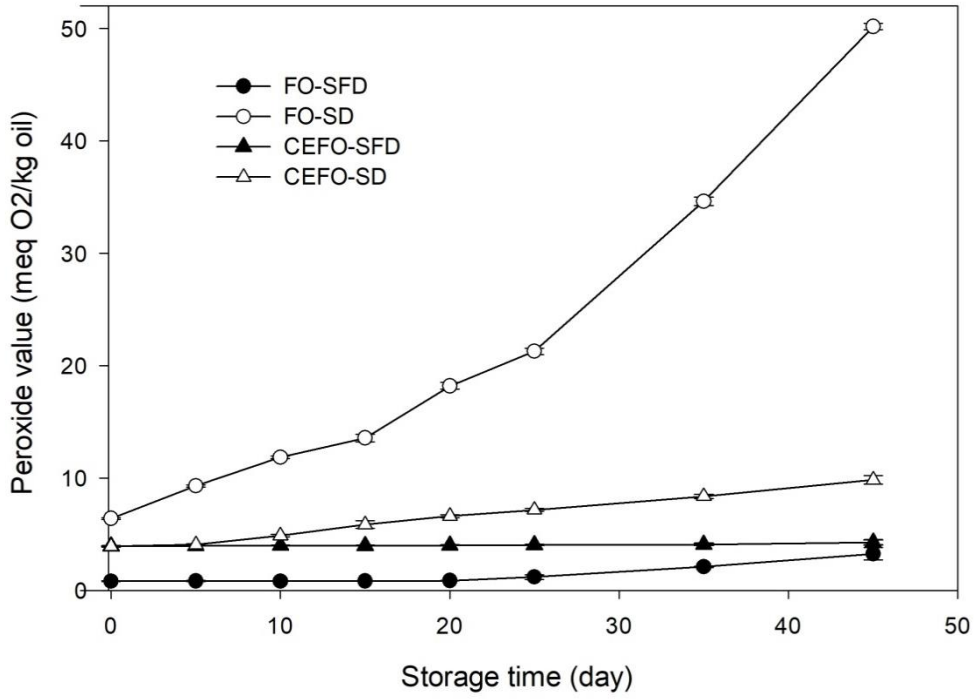


Figure 4.16 The effect of storage on PV in the encapsulated oil (FO: flaxseed oil; CEFO: carotenoid enriched flaxseed oil; SFD: spray freeze drying; SD: spray drying)

As it is seen in Figure 4.16, PV of CEFO-SFD powder increased from 3.96±0.01 to 4.26 ± 0.34 meq O_2/kg oil by the end of storage period of 45 days. The final PV of CEFO-SFD powder seems higher compared to that of FO-SFD powder (3.25 ± 0.21 meq O_2/kg oil). However, when their initial values have been considered, it can be easily understood that the rate of increase in PV value of FO-SFD powder is significantly (p<0.05) higher (4 times) than that of CEFO-SFD powder (1.1 times). Based on this information, CEFO-SFD powder was more stable to the oxidation reactions in terms of PV during the storage period. This oxidation stability behavior of CEFO-SFD powder is possible because of the available carotenoids in its composition. On the other hand, PV of CEFO-SD powder increased at a much higher level after 45 days (9.82 ± 0.53 meq O_2/kg oil) compared to that of CEFO-SFD. This difference could be explained with the effect of higher temperature used in SD process. Even if PV of CEFO-SD powder is higher than that of CEFO-SFD, it is still significantly (p<0.05) lower compared to FO-SD powder (50.18 ± 0.42 meq O_2/kg oil). These results indicate that carotenoids having the role of an important

antioxidant are effective to preserve the products even in the processes applied at higher temperatures.

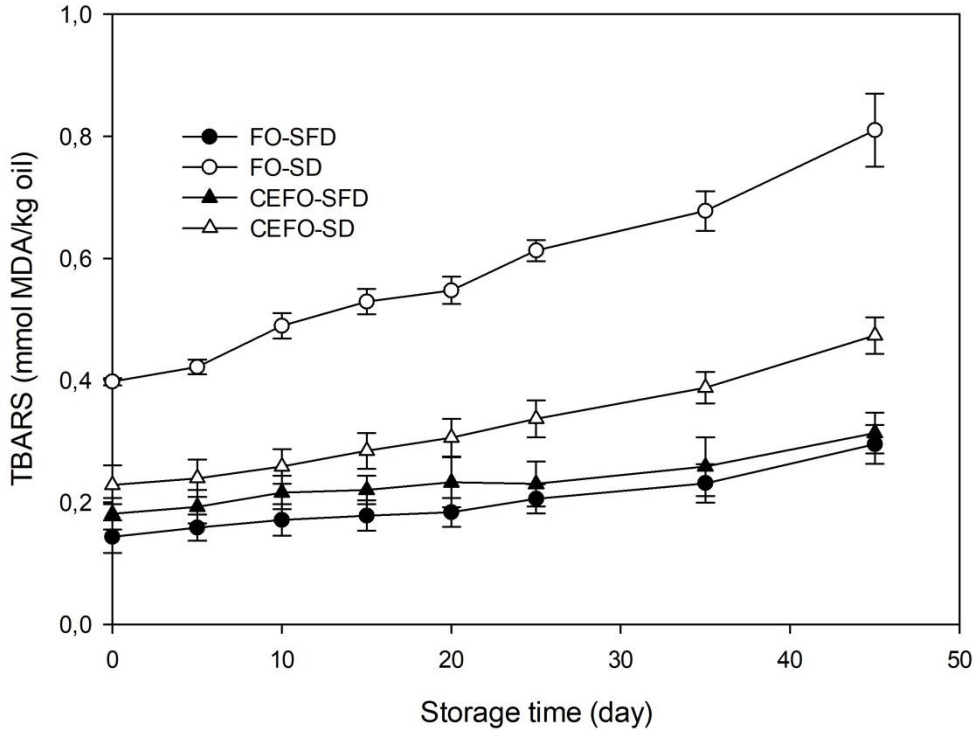


Figure 4.17 The effect of storage on TBARS values in encapsulated products (FO: flaxseed oil; CEFO: carotenoid enriched flaxseed oil; SFD: spray freeze drying; SD: spray drying)

Secondary oxidation products of the powders were determined by TBARS analysis. Although there is no legal limit for TBARS, it has been stated that the TBARS value should not exceed 1 mmol/kg oil (Fioramonti et al., 2019). Table 4.9 shows the effects of both carotenoid addition and encapsulation techniques on TBARS values of powders. The increase in TBARS value of FO-SFD powder is statistically insignificant (p> 0.05). On the other hand, TBARS value of FO appears to increase almost four times (from 0.11±0.02 to 0.40 ± 0.01 mmol/kg oil) when encapsulated by SD process. Although there is a significant increase in TBARS value after encapsulation by SD, it is still below the limit of acceptable rancidity level. In the encapsulation of CEFO, encapsulation techniques (SFD and SD) did not cause statistically significant increases in TBARS values. It was observed that SFD and SD techniques used for encapsulation of CEFO containing carotenoid, as in PV, did not lead to remarkable oxidative damage on the powder products obtained. Unlike FO,

which does not contain carotenoid, it has again shown that carotenoids in the oil also effective in reduction in formation of secondary oxidation products of the powder products even in encapsulation technique applied at high temperatures (SD).

Secondary oxidation products of encapsulated powders were monitored at 25°C for 45 days. TBARS values of the powders during the storage period are presented in Figure 4.17. TBARS values increased during storage in all powder products. The TBARS value of FO-SD powder reached 0.81 ± 0.06 mmol/kg of oil at the end of 45 days. As it can be seen from Figure 4.15, TBARS values of encapsulated powder products (FO-SD and CEFO-SD powders) obtained using the SD process increased faster during storage. This is probably due to their high initial PV and TBARS values. On the other hand, secondary oxidation products were formed at a lower level during storage of encapsulated products obtained by SFD processes. Carotenoids with the high antioxidant effect may have prevented the formation of secondary products which have been produced by decomposition of peroxides. Baik et al. (2004) also reported that the use of α-tocopherol as antioxidant significantly reduced the formation of secondary oxidation products during the storage of fish oil powder.

CHAPTER 5

CONCLUSION

In this thesis, it was aimed to encapsulate FO enriched by microwave technique by using innovative wall materials. In the first part of study, FO was enriched in carotenoids by microwave-assisted extraction. The simultaneous optimization of MACE was established for extracting carotenoids from CPW using FO as a solvent. The following conclusions can be drawn from this part of the study.

- It was observed that microwave power and extraction time are significantly effective in extracting carotenoids from CPW.
- The optimum conditions were determined as 165 W of microwave power, 9.39 min of extraction time and 8.06:1 g/g of oil to solid ratio.
- A 77.48 % recovery of carotenoid was achieved successfully at optimum conditions.
- It was concluded that microwave assisted extraction technique using the oil as a solvent can be regarded as a promising and efficient process for the extraction of carotenoids from the waste material from the production of carrot juice.
- In this way, an alternative method based on innovative and fast technology has been developed for both enrichment of oils and valorization of CPW in food industry.

In the second part of the study, the characteristics of emulsions containing pectin, wax, maltodextrin and CEFO were revealed. The following conclusions can be drawn from the results.

- The stability study revealed that most of the emulsions were kinetically stable after 24 hours.
- All emulsions showed very narrow and uniform droplet size distributions, indicating that there was no extensive aggregation between the oil droplets for all emulsions.

- MD, LMP and SFW used in the emulsification process indicated good emulsifying properties.
- Thus, the combination of MD, LMP and SFW could be an alternative way to improve emulsion stability.

In the last part of the study, CEFO was encapsulated by SFD using pectin, wax and maltodextrin as the wall materials. The following conclusions can be drawn from the results.

- CEFO concentration and MD/(LMP+SFW) ratio had significant impacts on encapsulation efficiency in SFD.
- Formulation with the highest encapsulation efficiency (70.9%) was 6% CEFO and 6:1 ratio of MD/(LMP+SFW).
- The encapsulation efficiency was 89.8% when the same formulation was processed by SD technique.
- Although lower encapsulation efficiency was acquired during SFD process, its oxidation stability was higher than SD process.
- The presence of carotenoids as well as the encapsulation technique remarkably contributed to the oxidative stability.
- In the light of the results, it can be said that SFD technique is a very suitable method for the encapsulation of oils that are sensitive to oxidation.
- Furthermore, the addition of an antioxidant substance such as carotenoid preserves the oil during processing and increases its storage stability in terms of oxidative rancidity.

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APPENDIX

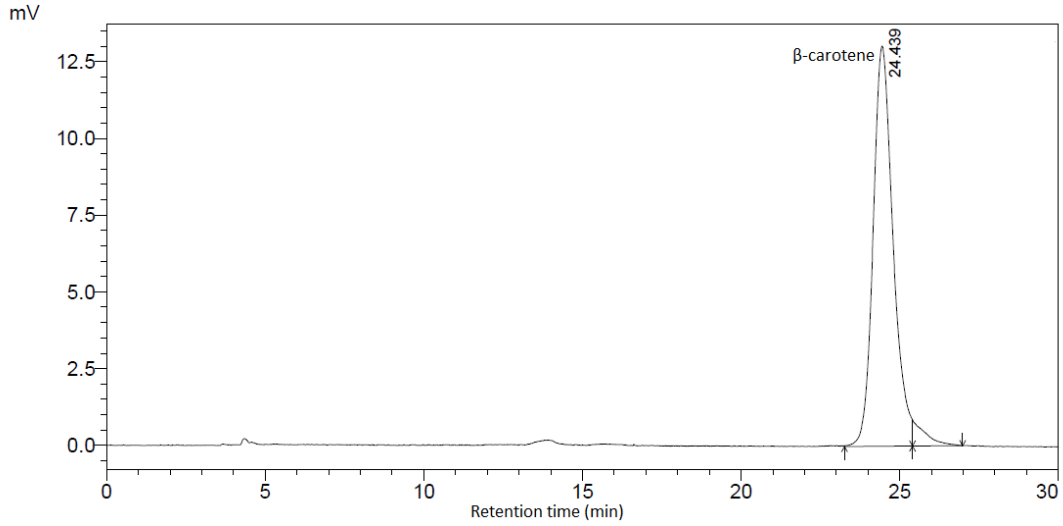


Figure A.1 HPLC chromatogram for β-carotene standard

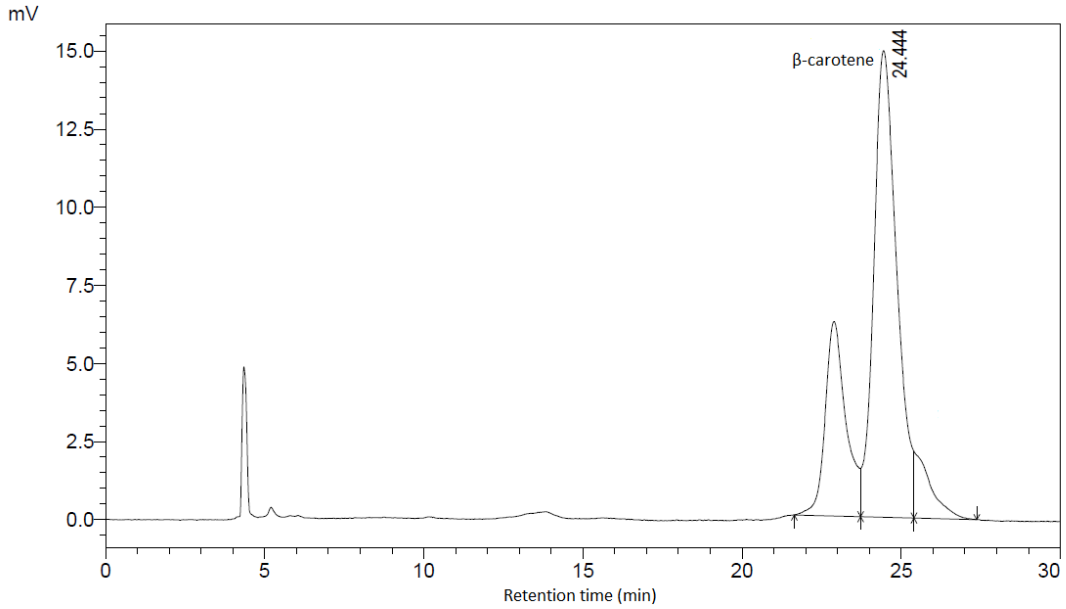
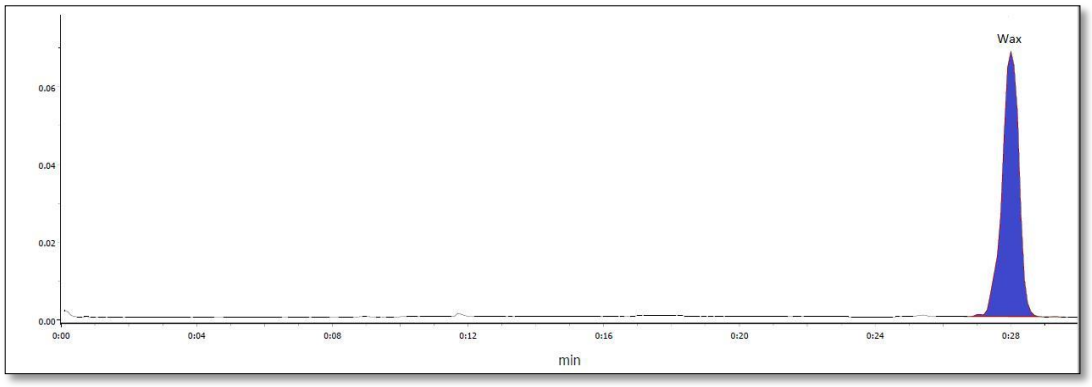
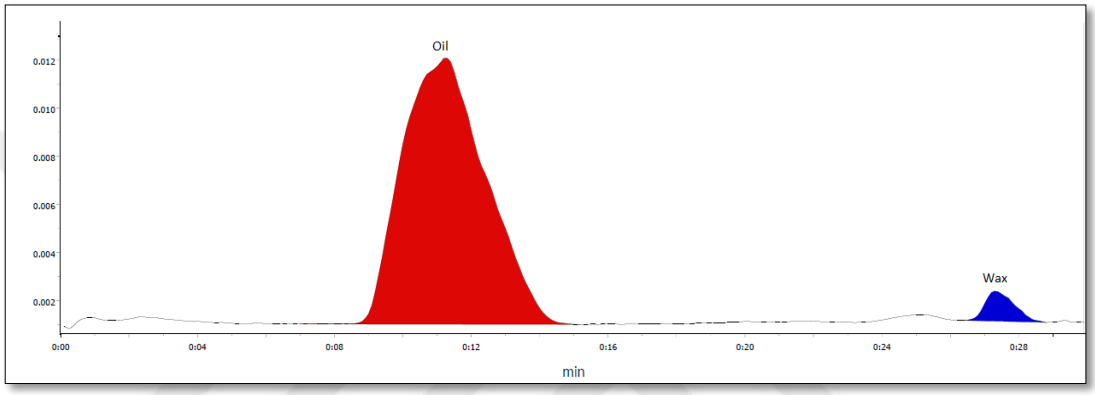


Figure A.2 HPLC chromatogram for CEFO



a



b

Figure A.3 TLC-FID chromatograms (a) sunflower wax; (b) surface oil

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B.Sc.	Ege University, Food Engineering Department	2006-2011

WORK EXPERIENCE

December 2013-Present	Gaziantep University, Food Engineering Department	Research Assistant

INTERNATIONAL/ACADEMIC VISITS

Year	Place	Enrollment
May 2019	Centro Tecnológico Nacional de la Conserva y Alimentación, Murcia, Spain.	Project Partner
September 2018	Interdepartmental Centre for Agrifood Industrial Research of Bologna University, Italy.	Training Stuff
March 2018-June 2018	BRISK 2 Project, Laboratório Nacional de Energia e Geologia (LNEG), Unidade de Bioenergia, Lisbon, Portugal.	Visiting Researcher
September 2014-January 2015	ERASMUS+, Kaunas University of Technology, Food Science and Technology Department, Kaunas, Lithuania	Visiting Researcher

PROJECTS

Year	Project Title	Project	Enrollment
2020-Present	Innovative Mild Processing Tailored to Ensure Sustainable and High Quality Organic Fruit Products	NH2020 SUSFOOD	Researcher

2018- Present	Pilot Scale Dried Apricot Production with Combined Solar and Radio Frequency System and Optimum Process Design: Pretreatment (sulfuring/extract application), Drying and Storage	TUBITAK 1003	Scholar
2018-2020	Encapsulation of flaxseed oil enriched in carotenoid by microwave technique using innovative wall materials	TUBITAK 1001	Scholar
2017-2019	Best Innovative Approach to Minimize Post Harvest Losses within Food Chain for VET	ERASMUS KA2	Researcher
2015-2018	ECOBERRIES-Ensuring Quality and Safety of Organic Food Along the Processing Chain	FP7 ERANET	Scholar

PUBLICATIONS

1. **Elik, A.,** Yanık, D. K., & Göğüş, F. (2020). Microwave-Assisted Extraction of Carotenoids from Carrot Juice Processing Waste Using Flaxseed Oil as a Solvent. LWT, 123, 109100.
2. Yanik, D. K., **Elik, A.,** Istanbullu, Y., & Göğüş, F. (2020). Fruits and Vegetables Purchasing Attitudes in Turkey: Packaging And Loss Reduction. International Journal of Scientific and Technological Research, 6(8), 39-45.
3. **Elik, A.**, Yanik, D. K., & Göğüş, F. (2019). Optimization of Microwave-Assisted Extraction of Phenolics From Blueberry. Romanian Biotechnological Letters, 24(1), 30-40.

4. **Elik, A.**, Yanik, D. K., Istanbullu, Y., Guzelsoy, N. A., Yavuz, A., & Göğüş, F. (2019). Strategies to Reduce Post-Harvest Losses for Fruits and Vegetables. International Journal of Scientific and Technological Research, 5(3), 29-39.
5. **Elik, A.**, Yanik, D. K., & Göğüş, F. (2017). Optimization of Microwave-Assisted Extraction of Phenolics from Organic Strawberry Using Response Surface Methodology. Harran Tarım ve Gıda Bilimleri Dergisi, 21(2), 143-154.
6. **Elik, A.**, Yanık, D. K., Maskan, M., & Göğüş, F. (2016). Influence of Three Different Concentration Techniques on Evaporation Rate, Color and Phenolics Content of Blueberry Juice. Journal of Food Science and Technology, 53(5), 2389-2395.

International Conference Papers Published in Proceedings

1. **Elik, A.**, Koçak Yanık, D., Göğüş, F. (2020). Packaging Opportunities of Fresh Tomato, 9th International Conference on Mathematics, Engineering & Natural Sciences (EJONS), Marrakech, Morocco.
2. **Elik, A.**, Koçak Yanık, D., Göğüş, F. (2020). Characterization of Oil-In-Water (O/W) Emulsions Containing Pectin, Wax, Maltodextrin, 9th International Conference on Mathematics, Engineering & Natural Sciences (EJONS), Marrakech, Morocco.
3. Işınay, B., Sever, M., Bulut, Ş. E., Özbek, H. N., **Elik, A**., Topçam, H., Erdoğdu, F., Koçak Yanık, D., Dalgıç, A. C., Göğüş, F. (2019). Effect of Combined RadioFrequency and Solar Assisted Air Drying on Properties of Dried Apricot, 3rd International Zeugma Conference on Scientific Researches, Gaziantep, Turkey.
4. Sever, M., Işınay, B., Bulut, Ş. E., Topçam, H., Özbek, H. N., **Elik, A.**, Göğüş, F., Dalgıç, A. C., Erdoğdu, F., Koçak Yanık, D. (2019). Drying of Whole Sulphured Apricots Using Pilot Level Solar Energy Assisted Air Drying and Radio-Frequency Finishing System, 3rd International Zeugma Conference on Scientific Researches, Gaziantep, Turkey.
5. Barış, S., **Elik, A.**, Koçak Yanık, D., Göğüş, F. (2019).Extraction of Pectin from Pistachio Green Hull, 3rd International Zeugma Conference on Scientific Researches, Gaziantep, Turkey.

6. **Elik, A.**, Koçak Yanık, D., Göğüş, F. (2019). Karotenoidlerin Atiklardan Geri Kazaniminda Yağlarin Ekstraksiyon Ortami Olarak Kullanimi, YABİTED 4. Bitkisel yağ kongresi, İstanbul, Turkey.
7. **Elik, A.**, Koçak Yanık, D., Göğüş, F. (2015). Extraction of phenolic compounds from strawberries using microwave technique, Biologically Active Compounds in Food, Lodz, Poland.

PRESS NEWS

- Postharvest project, Press New Titled "GAÜN'ün Postharvest projesi iyi uygulama seçildi" (İha, Habertürk, Haber 16, Net Haber, Zafer, Gazete Rize, Haber Alanya, İnegöl Online, Ege.Net, Gaziantep Gerçek Haber, Doğu Rehberi, Flikoston, Birebir Haber, Ekovitrin, Manşettürkiyem, Çamlık, Haber Mersin, Gaziantep Güneş, Telgraf, Talas Ekspres Haber, Beyaz Gündem, Sondakik Haber)
- Tübitak 1003 project, Press New Titled "Kurutulan meyve ve sebzenin rutubeti radyo frekansıyla azaltıldı" (Hürriyet, Tr Bülten, Çoban Web).
- Ecoberries project (FP7 ERANET), Press New Titled "GAÜN'ün Projesine AB'den Destek" (Hürriyet, Milliyet, Foodelphi, Haberler.com).

OTHERS

Elik, A., Koçak Yanık, D., Göğüş, F. (2018). Packaging Opportunities/Paketleme Olanakları, Postharvest Project Training Material, 96 Pages.