DEVELOPMENT OF HIGH STABILITY ANTIOXIDANT EMULSIONS BASED ON CITRUS AND FIG PECTINS

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ABSTRACT

DEVELOPMENT OF HIGH STABILITY ANTIOXIDANT EMULSIONS BASED ON CITRUS AND FIG PECTINS

In this thesis, stability and antioxidant activity of olive oil-in-water emulsions prepared with citrus pectin (CPEC) and pectins extracted from two fig cultivars (Sarılop: FPECn and Siyah Orak: FPECc) were evaluated in the presence of green tea (GTE) and grape seed extracts (GSE), and a basic monomeric flavonoid, (+) catechin (CAT). The emulsion stabilities of FPECn and FPECc between 0.125 and 1% (w/v) were comparable to those of CPEC. Control olive oil-in-water emulsions with 0.5% CPEC, FPECn or FPECc prepared without the addition of polyphenols lost 29 to 36% of their initial emulsion stability only within 1 day. The addition of GSE at 0.25 or 0.5% caused a considerable increase in the stability of emulsions prepared with CPEC at 0.5% (less than 10% loss in emulsion stability within 14 days) while GTE caused only a limited increase in the stability of CPEC emulsions. The CAT is the only polyphenol that caused significant increases in the stability of all pectin emulsions. In contrast, both GTE and GSE showed almost no effect in stability of FPECn and FPECc emulsions. The polyphenol added emulsions were characterized for their droplet size, zeta potential and viscosities. The CAT and GSE added emulsions showed significantly higher total phenolic content and antioxidant activity than GTE added emulsions during 14 days of storage. This thesis clearly showed that GSE and CAT stabilized CPEC emulsions, and CAT stabilized FPECn or FPECc emulsions have a great potential to develop novel antioxidant olive oil based functional foods.

ÖZET

TURUNÇGİL VE İNCİR PEKTİNİ TEMELLİ YÜKSEK STABİLİTE GÖSTEREN ANTİOKSİDANT EMÜLSİYONLARIN GELİŞTİRİLMESİ

Bu tezde turunçgil pektini (CPEC) ve bizzat iki farklı incir çeşitinden ekstrakte edilmiş pektinler (Sarılop: FPECn ve Siyah Orak: FPECc) yardımıyla oluşturulan su-içinde-zeytinyağı emülsiyonlarının yeşil çay ekstraktı (GTE), üzüm çekirdeği ekstraktı (GSE) ve monomerik yapıda temel bir flavonoid olan (+) kateşin (CAT) varlığındaki stabilitesi ve antioksidant aktivitesi incelenmiştir. FPECn ve FPECc'in %0.125 ve 1 (w/v) arasında oluşturduğu emülsiyonların stabiliteleri CPEC'in oluşturduklarıyla benzer bulunmuştur. CPEC, FPECn veya FPECc'in %0.5 (w/v) konsantrasyonda kullanımıyla elde edilen ancak polifenol içermeyen kontrol emülsiyonlarda başlangıç emülsiyon stabilitesinin %29-36 kadarı yalnızca 1 gün içerisinde kaybolmaktadır. %0.5 CPEC kullanılarak elde edilen emülsiyonlara %0.25 veya 0.5 oranında GSE ilave edilmesiyle oldukça stabil (14 günde %10'dan az stabilite kaybı gösteren) emülsiyonlar elde edilmiştir, ancak GTE ilavesi CPEC emülsiyonlarının stabilitesinde yalnızca sınırlı bir artış sağlamaktadır. Tüm pektinlerce hazırlanmış olan emülsiyonların stabilitesi CAT varlığında önemli düzeyde artmaktadır. Buna karşın, GSE ve GTE ilavesinin FPECn ve FPECc emülsiyon stabilitesine kayda değer bir etkisi olmadığı gözlenmiştir. Tüm emülsiyonlar damlacık boyutu, zeta potansiyeli ve viskozite yönünden karakterize edilmişlerdir. CAT ve GSE içeren emülsiyonlar 14 günlük depolama sırasında GTE içerenlere göre çok daha yüksek polifenol miktarı ve antioksidant aktivite göstermiştir. Bu tez çalışması GSE ve CAT kullanılarak stabilize edilmiş CPEC emülsiyonları, ve CAT kullanılarak stabilize edilmiş FPECn veya FPECc emülsiyonlarının zeytinyağı temelli fonksiyonel gıda üretiminde kullanılabileceğini göstermiştir.

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CHAPTER 1

INTRODUCTION

An emulsion includes two immiscible fluids that many tiny droplets of one is finely dispersed into other by means of a surface active substance known as emulsifier. There are numerous food grade emulsifiers which are currently being used in food industry. The emulsifiers can be divided into two major groups: 1) synthetic emulsifiers and 2) natural emulsifiers. The demand for natural foods has led the authorities to shift to natural ingredients in order to produce 'clean' labelled food products. Hence studies regarding natural emulsifiers are becoming more and more significant.

The natural compounds having emulsifying ability are saponins, proteins, phospholipids and polysaccharides. Plants are largest source of polysaccharides, thus, agro-industrial wastes of plant origin are frequently utilized into production of value added emulsion stabilizers such as pectin. Pectin, the most complex polysaccharide found in plant kingdom, is formed by three different fractions: homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). The HG, RG-I and RG-II are attached covalently to each other and form almost 65, 20-35 and 10% of pectin molecule in plants, respectively (Chandrayan 2018; Harding et al. 2017). The linear HG fraction forms the "smooth regions" of pectin molecule while the branched RG-I and RG-II form the "hairy regions". Some other minor pectic fractions also exist such as xylogalacturonan, apiogalacturonan, arabinan, galactan, arabinogalactan I and arabinogalactan II (Gawkowska, Cybulska, and Zdunek 2018). Commercial pectins obtained mainly from citrus peels (~85%) and to some extend apple pomace (~14%) are highly demanded by the food, nutraceutical, pharmaceutical and cosmetics industry primarily for their excellent gelling and thickening properties (Ciriminna et al. 2016; Gawkowska, Cybulska, and Zdunek 2018; Yemenicioğlu et al. 2020). The production of pectin from sugar beet pulp has also attracted an increased industrial interest since this pectin has unique emulsifying properties originating from its bound hydrophobic protein and ferulic acid contents that help pectin molecule to act as a surface active hydrocolloid (Pacheco et al. 2019; Siew and Williams 2008; Williams et al. 2005). The emulsifying ability of pectin extracted from various sources such as potato pulp (J. S. Yang, Mu, and

Ma 2018), pumpkin (Cui and Chang 2014), onion flesh (Neckebroeck, Verkempinck, Bernaerts, et al. 2021), mango peel (Deng et al. 2020), okra (Kpodo et al. 2018), etc. have been investigated. In this thesis along with standard citrus pectin, novel pectins extracted from two cultivars of sun-dried fig (*Ficus carica* L.) fruit, Sarılop the most common fig cultivar in Turkey, and Siyah Orak a newly breed dark-coloured fig fruit have been characterized for their emulsifying capacities with or without the presence of phenolic extracts and pure polyphenols.

Turkey is the largest fig producer in the world with 310 thousand tons of production in 2019 followed by Egypt (~225 thousand tons) and Morocco (~153 thousand tons) (Hasdemir 2021). Turkey, with its 85,500 metric tons of production in the 2020/21 season, is also the largest producer and exporter of sun-dried figs in the world (Hasdemir 2021). Common fig fruit is known as a good source of functional and bioactive compounds such as polysaccharides and flavonoids with many potential applications in food industry (Chen et al. 2015). The fig polysaccharides have been historically known as invaluable ingredients due to their functional and biological properties. Fig pectin is known not only as a good emulsifier (Çavdaroğlu, Farris, and Yemenicioğlu 2020; Çavdaroğlu and Yemenicioğlu 2022), but also as an antioxidant (Gharibzahedi, Smith, and Guo 2019) and a bioactive agent having immunomodulatory activity (Chen et al. 2015; Du et al. 2018). The dark coloured fig cultivars are known particularly with their high polyphenol content (Del Caro and Piga 2008). Although most pectins show good emulsifying capacity novel pectins such as sugar beet pectin have gained a particular importance since it contains a covalently bound surface active protein component (Siew and Williams 2008). The use of combination of different natural emulsifiers and modification of natural emulsifiers by covalent binding of surface active conjugates (mostly proteins) have also gained a popularity (Alba and Kontogiorgos 2020; Surh, Decker, and McClements 2006; Murayama, Rankin, and Ikeda 2021; Salminen and Weiss 2014; Xu et al. 2014). Moreover, recently Z. Liu et al., (2020) demonstrated that addition of free ferulic acid enhanced the oil-in-water emulsion stability of citrus, apple and sugar beet pectins. Jingna Liu et al., (2021) also improved the emulsifying activity and stability of citrus pectin by grafting different phenolic acids such as p-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, or gallic acid onto pectin via lipase-catalysed transesterification. Thus, these findings suggested that free or grafted phenolic acids could be used to enhance the emulsion stabilizing effects of pectins. However, data related to effects of pure

flavonoids and commercially important phenolic extracts rich in monomeric and polymeric flavonoids on emulsifying activity and stability of pectin are scarce.

Thus, this thesis aimed stabilization of olive oil-in-water emulsions prepared with citrus and fig pectins using green tea (GTE) and grape seed extracts (GSE), and a basic flavonoid pure catechin (CAT) for the first time. GTEs rich mainly in monomeric catechins (Jeong et al. 2004) and GSE rich mainly in monomeric catechins, and their oligomers (2-7 subunits) and polymers (8 to 24+ monomers) called proanthocyanidine (Kennedy, Matthews, and Waterhouse 2000) are the most extensively used phenolic extracts used in nutraceuticals and functional foods (Zhou, Wang, and Wang 2020). The antioxidant and antimicrobial activity as well as health benefits of GSE and GTE such as anticarcinogenic (Komori et al. 1993; Mohansrinivasan et al. 2015), anti-inflammatory (Carullo et al. 2020; Ohishi et al. 2016), antidiabetic (W. Tang et al. 2013; Tusher et al. 2021) effects have been proven by different studies. (+)-catechin, a monomeric catechin, is also a promising antioxidant since it could be produced in considerable amounts using callus and suspension-culture cells, especially from hypocotyls of *Pelargonium hydropiper* seedlings (Ono et al. 1998). Moreover, it was also demonstrated that some traditional Asian medicinal plants like gambir (*Uncaria gambir*) and bark of *Ulmus pumila* contain (+)-catechin as the main phenolic constituent (Anggraini et al. 2011; Cho et al. 2016). This thesis is original in that it is the first study related to emulsion stabilizing effect of flavonoid rich extracts and a pure major flavonoid. Moreover, this work serves highlighting the importance of sun-dried fig fruit pectin as a value-added functional ingredient or additive. Due to many different factors such as climate change, wrong agricultural practices and processing, a considerable part of sun-dried fruits is labelled substandard since they suffer from severe damage caused by insects, rotting, sun-scalding, split and torn, or excessive drying. Thus, outcomes of this thesis might be beneficial to understand value of fig pectin as a functional agent and to evaluate substandard fruits for pectin extraction.

CHAPTER 2

LITERATURE REVIEW

2.1. Emulsions and Natural Emulsifiers

Emulsions are colloidal systems that are formed by two immiscible fluids where one fluid is dispersed within the other fluid known as continues phase (McClements 2004; Santamaria-echart et al. 2021). Considering the distribution of liquid phases (usually oil and water) within each other emulsions may be separated into three main groups as depicted in Figure 2.1. which are oil-in-water (O/W), water-in-oil (W/O) and multiple emulsions (water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O)) (Friberg, Larsson, and Sjöblom 2004; Sweeta Akbari and Abdurahman Hamid Nour 2018). Emulsions are thermodynamically unstable systems and they are prone to phase separation because of the constant contact of oil and water molecules. For the stabilization of this unstable system usually emulsifiers are incorporated into the system (Sweeta Akbari and Abdurahman Hamid Nour 2018).

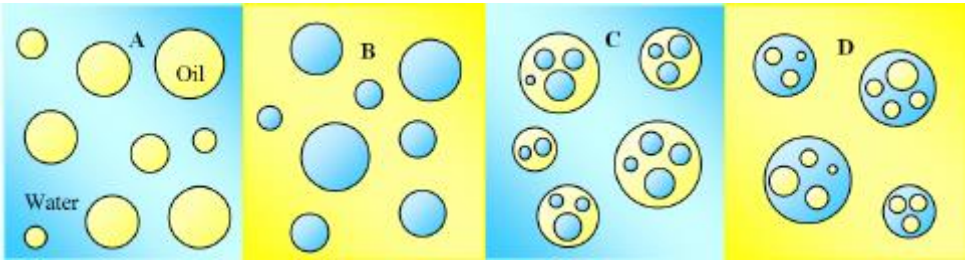

Figure 2.1. Schematic representation of a) oil-in-water (O/W), b) water-in-oil (W/O), c) water-in-oil-in-water (W/O/W), and d) oil-in-water-in-oil (O/W/O) emulsions (Source: Prichapan and Klinkesorn 2014)

Emulsions are essential in many industries such as food, chemical, biomedical, pharmaceutical and energy industries (Goodarzi and Zendehboudi 2018; Riess and Weers 1996). In food industry, most food products are formed of emulsions or have been in

emulsion state during their production such as mayonnaise, butter, ice cream, some fruit based drinks and salad dressings (McClements 2004). Emulsion systems are also preferred for encapsulation (Shah et al. 2016) and drug delivery (Frelichowska et al. 2009) purposes. The process of emulsion formation is known as *emulsification* and this process requires energy to break the dispersed phase liquid into small droplets. The required energy can be supplied into the system employing various devices such as mixers, ultrasound homogenizers, microfluidizers, colloid mill and membrane emulsification (Taha et al. 2020).

Effective emulsification process can be achieved by paid adsorption of emulsifiers to droplet surfaces so that interfacial tension can be rapidly decreased. In addition, surface activity of an emulsifier substance is significant. The emulsifier compound must contain both polar and non-polar groups within their structure and the ratio of these groups should be appropriate (Ozturk and McClements 2016). Once the emulsion formation is completed the stability of the system may result from the following mechanisms. First one is the electro-static repulsion between the charged droplets. As shown in the Figure 2.2a the charges present around the particles repulse each other and prevent coalescence (Sweeta Akbari and Abdurahman Hamid Nour 2018). Second mechanism is known as steric repulsion and occurs when an amphiphilic substance is present in the system that keeps the polar (water) and non-polar (oil) parts of the emulsion at a distance from each other as presented in Figure 2.2b. Another mechanism for the emulsion stabilization is the Marangoni-Gibbs effect. This phenomenon occurs when droplets get close to each other and the continuous phase of the emulsion forms a convective flux between them as represented in Figure 2.2c. During this movement some surfactant molecules also dislocates, leaving some parts with low concentration. The fluid will naturally move towards the low concentration because this movement is energetically favourable causing a protective layer around the droplets (McClements 2004). Final mechanism of stabilization occurs when there is a rigid or viscoelastic film covering the liquid droplets, this is also known as thin film stabilization, the system representation is given in Figure 2.2d (Sweeta Akbari and Abdurahman Hamid Nour 2018).

In the design of an emulsion-based food product the choice of the emulsifier plays a crucial role since the emulsion formation, stability and functionality highly depends on it. Emulsifiers can be studied under two classes. First class is the synthetic emulsifiers which are able to form excellent stable emulsions such as polysorbates, sorbitan esters

and sugar esters (Santamaria-echart et al. 2021; Dammak et al. 2020; Kim, Wang, and Selomulya 2020). However, there are some hesitations to use synthetic emulsifiers because they may cause various toxicities and intestinal inflammation (Singh and Ishikawa 2016; Lu et al. 2014).

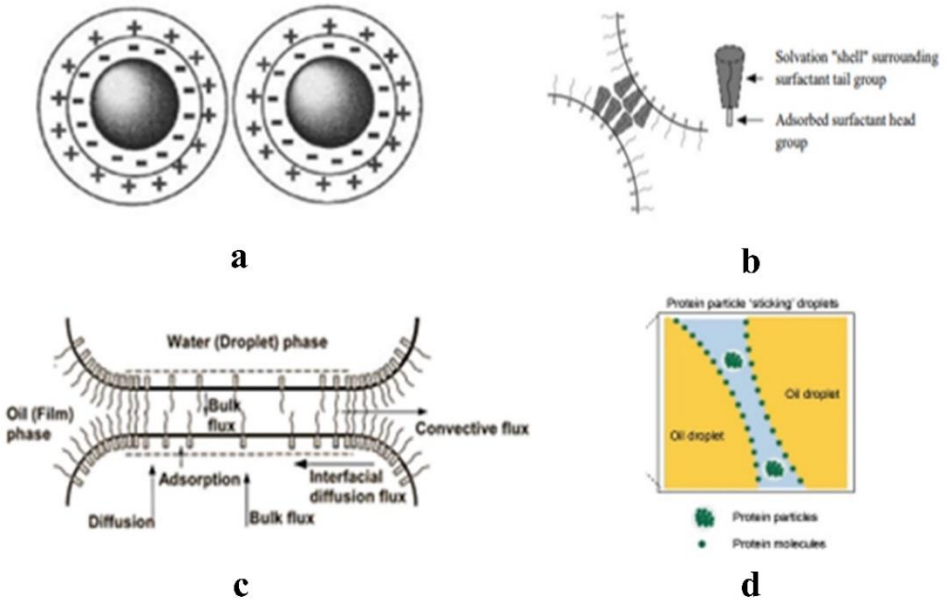

Figure 2.2. Schematic representation of a) electrostatic repulsion , b) steric repulsion, c) Maragoni-Gibbs, and d) thin-film emulsion stability mechanisms (Modified from: Sweeta Akbari and Abdurahman Hamid Nour 2018)

Second class is the natural emulsifiers and this class can be studied under four main chemical families which are proteins, saponins, phospholipids and polysaccharides (Ozturk and McClements 2016). The studies regarding characterization of these emulsifiers and evaluation of their functionality are gaining more attention every day. Since the consumer demand for natural food products is increasing, the producers are in search for the 'label-friendly' food emulsifiers that have good emulsifying capacity. Some products extracted from natural sources have proven themselves to be very good emulsifiers and commonly utilized in food industry. Main advantage of natural emulsifiers is that most of them have generally recognized as safe (GRAS) status. Although there are some limitations of natural emulsifiers currently more studies are being conducted upon the use of emulsifier blends or covalent conjugates (Surh, Decker,

and McClements 2006). The most common natural food-grade emulsifiers are individually reviewed in the following sections 2.1.1., 2.1.2, 2.1.3 and 2.1.4.

2.1.1. Proteins

Proteins are a group of natural emulsifiers that is commonly utilized in food industry. Their emulsification ability is due to their amphiphilic structure and depends on the source, structure, molecular weight and adsorption behaviour. Most commonly proteins stabilize emulsions with thin film stabilization mechanism. During emulsification process proteins are attached onto the surface of the droplets. After the attachment, the structure begins to unfold due to denaturation revealing the hydrophobic portion (Kim, Wang, and Selomulya 2020). The protein then adsorbs onto the particle surface and forms a thin viscoelastic layer that protects the emulsion from coalescence (Wilde et al. 2004). Protein extracted from dairy or plant sources can be used for emulsification purposes. Most common dairy proteins that are used as emulsifiers in food industry are casein and whey protein. These two differ in case of their structure while whey protein is a monomeric protein with globular structure, casein has a random coil structure that is more flexible (Kim, Wang, and Selomulya 2020). Because of the growing demand in plant-based foods, the applications of plant proteins as emulsifiers have also increased. There are numerous plant proteins that can be used for emulsification purposes such as legume, soy, pea, oilseed, wheat proteins. The proteins extracted from plants are mostly similar with each other and with whey protein in case of structure and they are all complex globular proteins. Casein on the other hand differs in case of its structure and it goes through more rapid changes compared to globular proteins since during emulsification process casein goes through a conformational realignment instead of denaturation (Kim, Wang, and Selomulya 2020). This process is more rapid and as mentioned earlier speed of attachment is an important factor in emulsion formation step. However, proteins' emulsification ability is highly dependent on environmental factors such as pH, temperature and ionic strength. In order to surpass this dependency nowadays combinations of proteins with other compounds are being studied. Most commonly protein-polysaccharide complexes are studied for emulsion formation. For instance, in a study conducted by Koupantsis and Kiosseoglou (2009) in order to surpass the effect of heat on whey proteins, the addition of carboxymethylcellulose was performed. The results showed that although droplet

flocculation still occurred, the effect of heating on the coagulation of proteins were decreased considerably.

2.1.2. Phospholipids

Phospholipids are one of the main constituents of a diverse group of natural sources such as milk, vegetable oils, egg yolk, meat and fish (Santamaria-echart et al. 2021). They are in amphiphilic nature and contain both hydrophilic part and a hydrophobic tail. As shown in Figure 2.3, phospholipid structure includes a polar phosphoric acid group and the hydrophobic section includes non-polar fatty acids. The amphiphilic property is responsible for phospholipids good emulsification ability. In addition, these substances also act as thickeners and they delay the phase separation in emulsions by increasing the viscosity of the medium. There are numerous phospholipids that are employed as emulsifiers in food such as phosphatidylcholine, phosphatidyglycerol, phosphatidic acid, phosphatidylethanolamine and phosphatidylinositol.

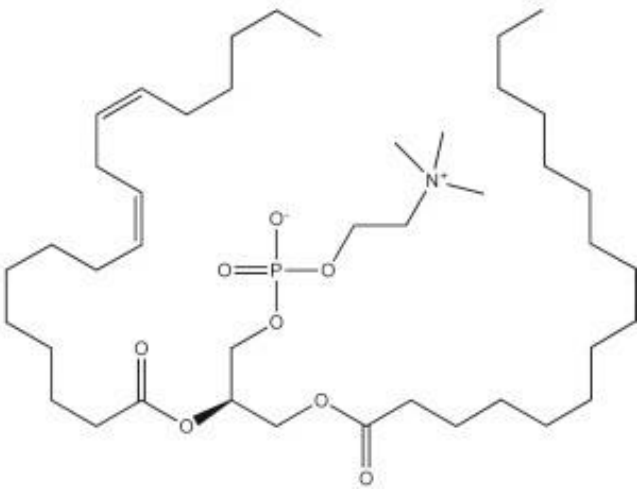

Figure 2.3. The general chemical formulations of lecithin (phosphatidylcholine) (Source: Chung et al. 2017)

The phospholipids utilized in food products are often attributed as lecithin which can contain several types of phospholipids (Ozturk and McClements 2016). Lecithin is abundantly used in food products such as mayonnaise and salad sauces as an emulsifier

due to its high emulsion stabilizing activity that resembles the surfactants. The solution pH and the nature of head group strongly influences the electrical charge of lecithin and may result in both negative and positive charges. The stability of lecithin emulsions is dependent upon the unsaturation degree of fatty acid chains because of the oxidation susceptibility of phospholipids (McClements and Jafari 2018).

2.1.3. Saponins

Saponins are a complex family of secondary metabolites which are found in more than 500 plant species (Reichert, Salminen, and Weiss 2019). They can be extracted from various plant sections such as leaves, seeds, roots, fruits and stems of chickpea, spinach, sugar beet, oats and *Quillaja saponaria* Molina trees. Saponin chemical structure varies extensively however they can be distinguished by the presence of steroid or triterpene sugar free aglycone that is known as sapogenin. They also contain a hydrophilic moiety which is sugar. The presence of both hydrophobic backbone and hydrophilic moiety improves the emulsification capacity of saponins. Almost always the saponins extracted from *Quillaja saponaria* Molina trees are used in food industry. These *Quillaja* saponin extracts contain quillaic acid groups and rhamnose, galactose or glucuronic acids as seen in Figure 2.4.

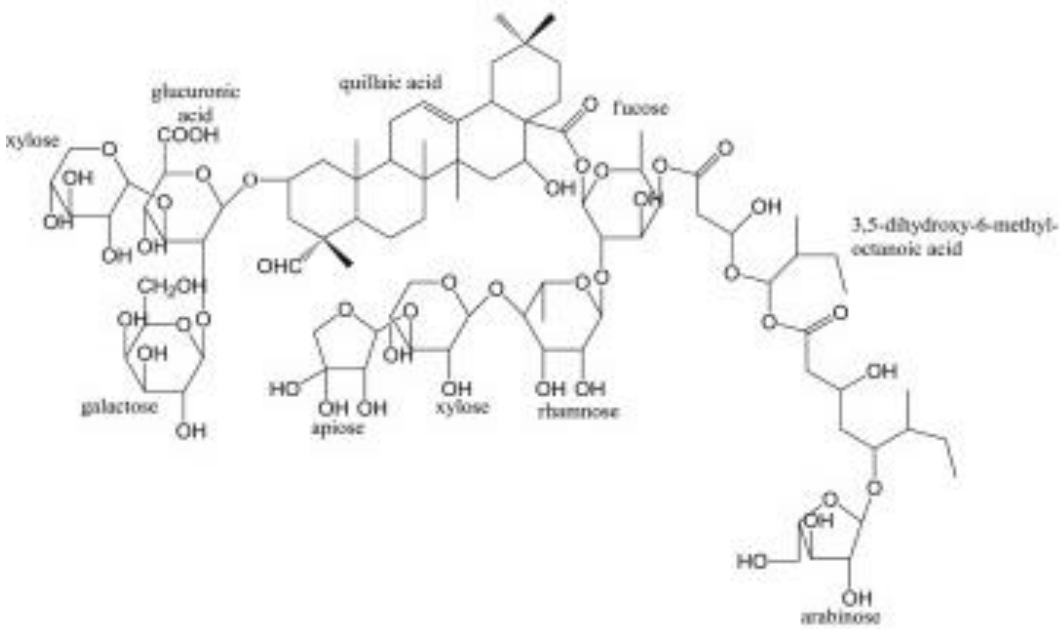

Figure 2.4. The chemical representation of *Quillaja* saponins
(Source: Chung et al. 2017)

They are able to produce highly stable emulsions that are mostly unaffected of environmental conditions such as pH, temperature and ionic strength (Kim, Wang, and Selomulya 2020). When the natural emulsifier *Quillaja* saponin and synthetic emulsifier Tween 80 was compared in case of their emulsification ability. It was determined that *Quillaja* saponin as an emulsifier is a promising natural replacement with the ability to form nano-emulsions (d < 200 nm), although the droplet size values obtained with Tween 80 (d < 150 nm) were smaller under the same conditions (Y. Yang et al. 2013).

2.1.4. Polysaccharides

Polysaccharides are among the most abundantly found nontoxic and natural biopolymers. They are complex molecules composed of monosaccharides bound by glycosidic linkages (Song, Shang, and Ratner 2012). Polysaccharide molecules may be composed of only one type of monosaccharide building block (homopolysaccharide) or various types of monosaccharides (heteropolysaccharide) with linear or branched structure (Pawar et al. 2008). Polysaccharides with varying sources and structures are often utilized in food industry because of their gelling, thickening, stabilizing and emulsifying ability. Their physical properties such as viscosity, water solubility and gelling behaviour is dependent upon the degree of branching (Stephen, Phillips, and Williams 2006). Polysaccharides can be divided into two main groups as starch-derived products and non-starch polysaccharides. The most commonly utilized non-starch polysaccharides in food industry include alginate, carrageenan, agar, gum arabic, xanthan gum, and pectin (Evans, Ratcliffe, and Williams 2013; Stephen, Phillips, and Williams 2006).

2.1.4.1. Pectin

Pectins are hydrocolloidal polysaccharides found in plant cell walls of higher plants. Middle lamella of plants contains the highest concentration of pectins followed by primary cell wall to plasma membrane (Thakur et al. 2009). They function as a cementing material within the plant cell and as a hydrating agent. Pectins are composed mainly of α-(1 → 4)-D-galacturonic acid (GalA) and they have four main structural components which are homogalacturonan (HG), xylogalacturonan (XGA), rhamnogalacturonan I

(RG-I) and rhamnogalacturonan II (RG-II) (Milošević and Antov 2022; Li et al. 2021) (Figure 2.5). The pectin molecule is formed with α-(1 → 4) glycosidic linkages among GalA units. The HG is the linear backbone of the pectin molecule which is described as smooth region. This portion of the pectin molecule is a homopolysaccharide with repeating units of GalA that may be partially methylated at *C-6* or acetylated at *O-2* and/or *O-3* (Ngouémazong et al. 2015; Morris et al. 2010). The rhamnogalacturonan sections, RG-I and RG-II, are responsible for the complexity of pectin molecules (Thakur et al. 2009). The RG-I portions of the pectin, also known as the hairy region, are the heterogeneous branched pectic sections composed of alternating unit [→2)-α-L-rhamnose-(1→4)-α-D-GalA-(1→] with various side chains (Morris et al. 2010; Ngouémazong et al. 2015).

Pectin plays a key role in the determination of fruit and vegetable quality in food industry since its structure changes with food processing operations. The endogenous enzymes pectin methyl esterase (PME) and polygalacturonase (PG) may interact with pectin when subjected to food processing conditions (Jianing Liu et al. 2020). These enzymes cause pectin depolymerization by hydrolysing the α-(1 → 4) glycosidic linkages of HG.

Having features such as emulsifier, gelling agent, thickener and stabilizer, pectin is commonly utilised in food, cosmetics and pharmaceuticals industry for its functional and health-promoting effects (Çavdaroğlu and Yemenicioğlu 2021; Gilani et al. 2008; Dimopoulou et al. 2019; Lal et al. 2021).

Commercially available pectin is obtained from citrus peel (lemon, lime and grapefruit), apple pomace or sugar beet pulp and studies show that sugar beet pectin (SBP) has unique emulsifying capacity due to its high protein and ferulic acid composition when compared to other standard pectin with linear backbone (Siew and Williams 2008; Lin et al. 2020). Commercial pectin are classified according to their esterification degree (DE) as high-methyl esterification pectin (HMP) with DE > 50 or low-methyl esterification pectin (LMP) with DE < 50 (De Azeredo et al. 2014). As the esterification degree changes the gelation mechanism for pectins changes. The LMP forms gels with the presence of divalent cations such as Ca^{2+} ions through a mechanism known as egg-box model while gelation of HMP occurs in the presence of a co-solute, usually sucrose, at low pH (Chan et al. 2017; Cao et al. 2020; Capel et al. 2006).

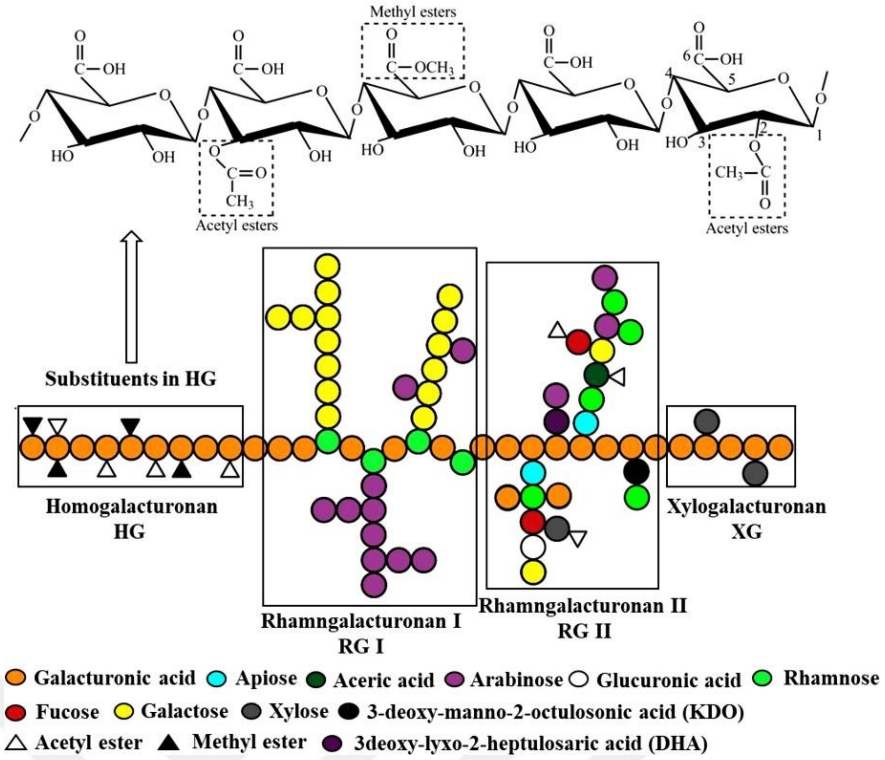

Figure 2.5. Schematic representation of pectin structure with its main components. (Source: Jianing Liu et al. 2020)

Recently, pectin is attracting considerable interest regarding its emulsifying properties since it is able to increase the stability of emulsions by forming steric and electrostatic interactions that prevents coalescence of oil droplets or by increasing the viscosity of the solution (Verkempinck et al. 2018; Mendez et al. 2021; Neckebroeck, Verkempinck, Van Audenhove, et al. 2021). Although the exact mechanism of emulsion formation and the chemical interactions are yet to be discovered there are many hypotheses regarding the possible reason for some pectin to show good emulsification capacity when some do not. The study performed by Leroux et al., (2003) concluded that the methylation degree of citrus pectin effects its emulsification capacity and the highest methoxylated citrus pectin showed the best emulsification capacity. They also obtained that the acetylation degree effects the emulsifying function of pectin possibly by reducing flocculation (Leroux et al. 2003). On the other hand, Siew & Williams, (2008) demonstrates emulsification capacity is related with the presence of hydrophobic groups such as proteins or ferulic acid within the pectin structure. Sugar beet pectin shows good emulsifying ability because it has both hydrophilic (polysaccharide chain) and hydrophobic (feruloylated and acetyl groups) parts in its structure (Niu et al. 2022).

The emulsifying ability of pectin extracted from various sources such as potato pulp (J. S. Yang, Mu, and Ma 2018), pumpkin (Cui and Chang 2014), onion flesh (Neckebroeck, Verkempinck, Bernaerts, et al. 2021), mango peel (Deng et al. 2020), okra (Kpodo et al. 2018) and many more have been investigated. Although these pectins have good emulsifying capacity, they were not able to stabilize emulsions as well as sugar beet pectin or proteins. In order to improve the functionality and the stability of the emulsions, emulsifier mixtures are investigated. Most commonly pectin-protein conjugates have been studied (Salminen and Weiss 2014; Murayama, Rankin, and Ikeda 2021; Xu et al. 2014). However, there are only limited data about effects of free and bound phenolic compounds in activity and stability of pectin stabilized emulsions. For example, Liu et al., (2020) recently demonstrated that addition of free ferulic acid enhanced the oil-in-water emulsion stability of citrus, apple and sugar beet pectins. These workers reported that the addition of ferulic acid reduced the sizes of oil droplets in pectin stabilized emulsions. The addition of ferulic acid also increased the amount of citrus and apple pectins absorbed at the oil-water interface (X. Liu, Le Bourvellec, and Renard 2020). Liu et al., (2021) also improved the emulsifying activity and stability of citrus pectin by grafting different phenolic acids such as *p*-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, or gallic acid onto pectin via lipase-catalysed transesterification. Currently, data related to effects of pure flavonoids and commercially important phenolic extracts rich in monomeric and polymeric flavonoids on emulsifying activity and stability of citrus pectin are scarce.

2.2. Fig Fruit (*Ficus carica* L.)

One of the most known members of the Moraceae family is the common fig tree (*Ficus carica*) and they are cultivated throughout the Mediterranean, Middle Eastern and other areas of the world with similar climate such as Turkey, Egypt, Iran, Pakistan, India and so on. The largest fig producer around the world is Turkey with ~310 thousand tons of production followed by Egypt with ~225 thousand tons and Morocco with ~153 thousand tons of fig production (Hasdemir 2021). Figs are rich in fiber, potassium, calcium and iron (Crisosto et al. 2011). Most commonly observed fig varieties include ‘Sarılop’, ‘Bursa Siyahı’, ‘Yediveren’ and ‘Morguz’ figs (Çalişkan and Polat 2008), additionally to these cultivars the ‘Siyah Orak’ cultivar is a relatively new and promising type of fig for

fresh consumption. Although, the Siyah Orak species is a relatively new one, currently its plantation is being popularized to extend the seasonal market time of figs on shelves. As Ertan (2016) states this species is relatively more durable than others, with its increasing production the use of this species will become widespread. Also, the black colour of this fig species suggests the presence of high levels of anthocyanins which are the pigments responsible for purple-black colour of fruits (Ercisli et al. 2012) (Figure 2.6b). These anthocyanins are known for their health promoting effects which should contribute to the spread of this fig variety. This fig species is preferred for fresh consumption while the 'Sarılop' cultivar is mostly retailed as dried products either for direct consumption or for use as an ingredient in other food products such as cereals. The Sarılop dried figs have softer texture, more natural colour, honey flavour and desirable odours when compared to dried figs from Iran and Italy (Arpacı, Konak, and Çiçek 2018) (Figure 2.6a). Hence this species is the leader in the world market for dried figs. The dried figs are also a good source of bioactive compounds (phenolics, phytosterols, coumarins, anthocyanins etc.). Both fresh and dried figs are known to have high phenolic and soluble dietary fibre contents. The fig fruit is quite perishable in its fresh from hence many producers dry their products usually by sun-drying method. The sun-drying process results in a significant amount of sub-standard fig fruits which are considered as agro-waste. Additionally, the stalks of the dried fig fruits can also be considered as waste material since they are required to be separated from the fruit prior to further processing.

a **b**

Figure 2.6. The fig variaties used in this study; a) Sarılop cultivar, and b) Siyah Orak cultivar (Source: Ek 2011)

A recent study by Çavdaroğlu & Yemenicioğlu, (2022) extracts pectin from both fig stalks and substandard whole figs (Sarılop cultivar) and determines that stalk pectin was a good source of pectin when the substandard fig pectin was only a moderate source.

2.3. Phenolic Compounds

Phenolic compounds are ubiquitous phytochemicals found in most fruits and vegetables because they are plant's response to external threads such as insect attack (Khoddami, Wilkes, and Roberts 2013). They are the secondary metabolites produced biogenetically from the shikimate or phenylpropanoid pathways in plants. The structure of simple phenolic compounds possesses an aromatic ring with one or more hydroxyl groups in its structure (Figure 2.7a) while the term 'polyphenol' indicates there are two or more phenyl rings present within the molecule structure (Figure 2.7b).

Due to their redox properties phenolic compounds exhibit remarkable antioxidant activity both *in vivo* and *in vitro* models (Mustafa et al. 2010; Shahidi et al. 2016) possess many health promoting effects including anticancer, anti-inflammation, antimicrobial and immune system regulating activity (Tungmunnithum et al. 2018). The presence of hydroxyl groups on the phenol structures ensures toxicity of phenolics against microorganisms (Alara, Abdurahman, and Ukaegbu 2021).

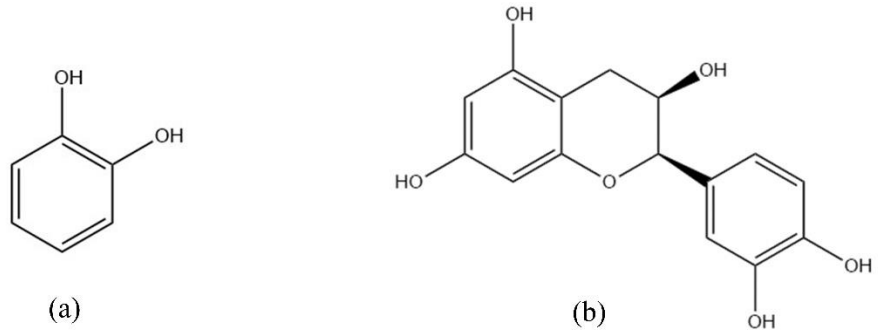

Figure 2.7. Chemical structure of a) simple phenolic compound containing only one phenol unit, and b) polyphenol compound containing multiple units (Figure drawn by the ChemDraw 18.1 software)

Phenolic compounds are divided into sub-classes according to their basic skeleton. Some common phenolic compounds include; simple phenol (C_6), phenolic acid (C_6-C_1),

flavonoids (C_6-C_3-C_6) and condensed tannins $(C_6\text{-}C_3\text{-}C_6)_n$. Additionally, the structures of polyphenols effect their solubility and separation properties. For instance, the phenolics with high molecular weight are usually insoluble (Alara, Abdurahman, and Ukaegbu 2021). The two largest groups among phenolic compounds are the flavonoids with almost half of the phenolics followed by phenolic acids are examined in more detail in the following sections 2.3.1 and 2.3.2.

2.3.1. Phenolic Acids

The phenolic acids are one of the most common phenolic compounds found in plants. It is very rare to find phenolic acids in their free form, usually they are found in nature in the form of esters, glycosides or amides (Khoddami, Wilkes, and Roberts 2013). The phenolic acids are formed by the derivation of either one of the parent chemicals which are hydroxycinnamic and hydroxybenzoic acid. The common phenolic acids *p*-coumaric, ferulic, caffeic acids are derived from hydroxycinnamic acid when the gallic, vanillic and syringic acids are derived from the hydroxybenzoic acid. The differences among phenolic acids are due to the substitution and functional groups such as hydroxyl and methoxy groups.

2.3.2. Flavonoids

Flavonoids, C_6-C_3-C_6, are the most common phenolic compounds in human diet. Their structure possesses three-rings, marked as A, B, and C, and they are formed by the derivation of aromatic amino acids, phenylalanine and tyrosine (Figure 2.8) (Khoddami, Wilkes, and Roberts 2013). The flavonoids are separated into 6 main groups which are flavones, flavanones, flavonols, catechins (flavan-3-ols), anthocyanins and isoflavones. Flavonoid compounds usually exist in their glycoside form where a sugar moiety binds to the hydroxyl group present in the structure except for catechin (Nishiumi et al. 2011).

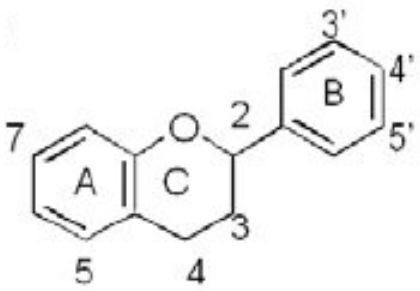

Figure 2.8. The chemical representation of flavonoids
(Modified from: Nishiumi et al. 2011)

Catechin can be found in two steric forms which are (+)-catechin and its enantiomer, but most food products contain the (+)-enantiomer of catechin (Figure 2.9.) (Donovan et al. 2006). Donovan et al., (2006) showed that the catechin absorption through small intestine was significantly higher than its (-)-enantiomer. Hence, (+)-catechins are used as antioxidative agents in oils and fats also in other food products due to their antimutagenic and antimicrobial properties (Yilmaz 2006). This monomeric flavonoid is a promising bioactive compound since significant amount of production by suspension-culture cells, especially from hypocotyls of *Pelargonium hydropiper* seedlings are possible (Ono et al. 1998). Additionally many medicinal plants contain catechin in high amounts such as gambir (*Uncaria gambir*) and bark of *Ulmus pumila* which are promising novel catechin sources (Anggraini et al. 2011; Cho et al. 2016).

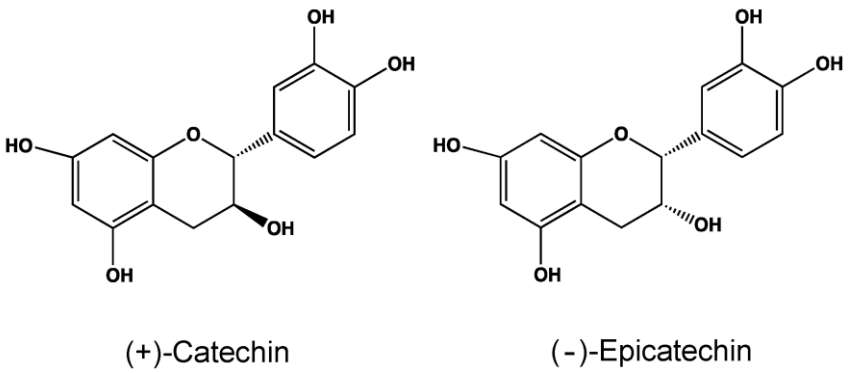

Figure 2.9. The chemical representation of (+)-catechin and its enantiomer
(Modified from: Isemura 2019)

2.3.3. Phenolic Extracts

Phenolic compounds extracted from the plants are named as phenolic extracts which usually exhibit good bioactive properties. Two of the most commonly utilized phenolics extracts are the grape seed (GSE) and green tea (GTE) extracts due to their antimicrobial and antioxidative effects (Zhou, Wang, and Wang 2020). In addition to their popularity due to the shift to natural replacements of synthetic food additives, these phenolic extracts show more antioxidative activity than their synthetic substitutes (Namal Senanayake, 2013).

Green tea is a commonly utilized as the phenolic extract source due to its high phenolic compound ratio when compared to other teas such as oolong or black tea (Namal Senanayake, 2013). The GTE contain numerous phenolic compounds but the main bioactive moiety of GTE is the monomeric flavonoids such as catechins, epicatechins, epicatechin gallate, epigallocatechin gallate etc (Chacko et al. 2010). Due to its high phenolic content GTE is often utilized to increase the shelf life of lipidic food products by preventing or delaying the lipid oxidation. Similar to GTE, GSE also contains significant monomeric phenolics (catechins), its main bioactive components are the oligomeric proanthocyanidine (Yilmazer-Musa et al. 2012). The GSE is commonly utilized in food industry due to its antimicrobial, antioxidant and antidiabetic properties (Su and D'Souza 2011).

These phenolic extracts' effect on health is studied in numerous animal models including rat, mice and rabbit (Duong et al. 2016). A study conducted by Zhou et al., (2020) obtained that the GTE shows higher antioxidant activity when compared to GSE which is probably due to the hydrophilicity of GTE molecules. Their effect on the lipid oxidation was studies by Rababah et al., (2011) and both plant extracts were found to significantly effective on minimizing of lipid oxidation in meat samples. Additionally, the redness of the meat samples was evaluated and while GTE treated samples showed redness, in GSE treated samples redness was significantly higher (Rababah et al. 2011). The redness in meats is a critical quality attribute since the decrease in redness may point to the oxygenation of meat myoglobin.

2.4. Olive Oil

The oil extracted from the olive fruit (*Olea europea* L.) is described as olive oil (OO). The extraction method and condition of oils from the fruits determine the OO classification. The extracted OO can be separated three main classes which are extra-virgin olive oil (EVOO), virgin olive oil and refined olive oil. These OOs differ highly in case of their chemical composition. The EVOO composition includes 97-99% triglycerides and 1-3% minor compounds (Jimenez-Lopez et al. 2020), other characteristics includes the presence of higher amount of phenolic compounds (most commonly *p*-coumaric, ferulic, gallic, vanillic and caffeic acids) (Ambrosewicz, Tańska, and Rotkiewicz 2012) and having lower free acidity (≤ 0.8) (Peri 2014) than other OO classes. The EVOO has higher mono- to poly-unsaturated fatty acid ratio hence they are more resistant to oxidation than the other OO classes (Cinelli, Cofelice, and Venditti 2020) and EVOO contain high amounts of oleic acid which is used as a surfactant in functional nanoparticles (Shahruzzaman et al. 2022).

The epidemiological evaluation of Mediterranean regions showed that the occurrence of some cancer types and coronary heart diseases are decreased in these countries (Erbay and Icier 2010). Inevitably this phenomenon was linked to the Mediterranean diet which is rich in fruits, vegetables and olive oil. These food products are rich in phenolics and vitamins while the OO is especially a phenolic rich food product. The known health promoting effects of these phenolics partially ties the healthy lifestyle of Mediterranean people to their olive oil consumption (Badimon and Perez Jimenez 2005). Additionally, use of monounsaturated fatty acid rich oils such as EVOO was shown to decrease the curbing weight gain in men that consumes high-fat diets (Piers et al. 2002). Another study reveals that EVOO intake enhances insulin sensitivity and regulates glucose homeostasis in high-fat diet mice model for Type 2 Diabetes (Jurado-Ruiz et al. 2019). The use of EVOO is also preferred for technological purposes in emulsion based foods because of their lower unsaturation higher antioxidant properties (Mosca et al. 2013; Sun et al. 2011).

2.5. The Effects of Antioxidants on Health and Well-being

Antioxidants are materials that delay lipid oxidation by two main mechanisms which are the inhibition of free radical formation and by interfering with the production of more free radicals to stop the oxidation reaction (Namal Senanayake, 2013). Antioxidants are the main ingredients that prevent oxidative deterioration of lipids to ensure food quality. The oxidative stress in addition to decreasing the nutritional value and quality of foods, plays a key role in the occurrence of numerous conditions such as inflammation, cataract, cancer, aging and Parkinson's disease (Dias, Junn, and M. Maral 2013; Martins, Barros, and Ferreira 2016). Antonicelli et al., (2004) found that nacystelyn, an antioxidant compound, alters the redox balance in the lungs and lower the inflammation. Similarly, Zhang et al., (2003) studied antioxidant activity of a polysaccharide fraction from *Porphyra haitanesis* in case of its effects on aging in mice. The results showed anti-aging effect of this compound achieved by the compensation of reduced antioxidative activities in cells (Zhang et al. 2003).

The antioxidants can be naturally sourced or synthetic and both types are highly effective in case of reducing oxidation in products. However, due to safety concerns there is a legal limitation for synthetic antioxidant usage in foods hence one of the most promising natural antioxidant source which are plant phenolic compounds became popular (Shahidi and Ambigaipalan 2015). Phenolic compounds especially flavonoids are known for their antioxidant activity. They show antioxidant activity by acting as free radical acceptors, this mechanism also known as reactive oxygen species scavenging ability (Higdon, Frei, and Blumberg 2003). They rapidly donate a hydrogen atom to radicals hence preventing the oxidation lipids. Phenolic compounds are ideal antioxidants because they contain hydroxyl groups within their structure that are prone to donating an electron or a hydrogen atom to the free radical. Secondly, they have extended conjugated aromatic system to delocalize an unpaired electron (Dai and Mumper 2010). Phenolic compounds also show antioxidative activity through metal chelation where the metals are chelated by the antioxidant substance thus preventing the metals to further catalyse the free radical formation (Frei and Higdon 2003). Other antioxidant activity mechanisms include the inhibition of redox-sensitive transcription factors such as kinase in animal models and the inhibition of pro-oxidant enzymes (Frei and Higdon 2003). In the literature there is an abundancy regarding the antioxidative activity of phenolic compounds when there are

only some *in vivo* studies conducted and the clinical trials in this field are very scarce. According to Martins et al., (2016), there are significant differences between *in vitro* and *in vivo* studies for antioxidant activity of phenolics. Hence more *in vivo* and clinical studies regarding antioxidants and their mechanism of action are required.

Epidemiological studies show the health promoting effect of phenolics once they are taken into the body. Their journey begins with the consumption of complex food matrix which travels through the digestive track and reach to the small intestine. In the small intestine, the phenolics are taken into the body through the epithelial cells and instantaneously they form conjugates with other materials such as glucuronic acid (Shahidi and Yeo 2018). This study determined the absorption is only limited to 5-10% in the small intestine (Shahidi and Yeo 2018).

The phenolic compounds in general have low solubility, low stability and low bioavailability, many research focus on improving their stability and bioavailability which shows that very limited amount of the bioactive compound, phenolics, are able to enter the bloodstream. Another limitation is that usually the phenolic compounds are linked to soluble polysaccharides which are indigestible in the small intestine (Wang et al., 2014). There are many strategies developed to overcome these barriers and increase the bioaccessibility and bioavailability of phenolic compounds. For some of the phenolic compounds, mostly phenolic acids, increase in bioaccessibility increases their bioavailability. Thus, processing steps such as milling, microfluidization, thermal treatment, extrusion cooking and bioprocessing that separates the indigestible polysaccharides and phenolics are employed to overcome this phenomenon (Wang et al., 2014).

The phenolic compounds such as curcumin, quercetin, resveratrol etc. are lipophilic compounds and the presence of hydrophobic environment increases their solubility hence the bioavailability increases. Mostly, the lipidic environment is achieved by emulsion/nano emulsion where they can be used as liquid systems or dried to form powders. One of the most established methods for the phenolic compounds with low solubility and stability is the encapsulation of phenolics using lipid-based carriers.

CHAPTER 3

MATERIALS and METHODS

3.1. Materials

Citrus pectin with a degree of esterification between 50 and 75% and galacturonic acid content at ≥ 74% was obtained from Sigma Aldrich Co., Ltd (İzmir, Turkey). Phenolic extracts used in the study were supplied from; Polyphenolics (CA, USA) for grape seed extract (GSE), Wild (Eppelheim, Germany) for green tea extract (GTE) and Sigma Aldrich Co., Ltd (İzmir, Turkey) for catechin (CAT). The commercial extra virgin olive oil used for emulsions was purchased from local markets same batch was used throughout the study without further purification. The sun-dried Sarilop figs were supplied by KFC Gıda Tekstil Sanayi İthalat İhracat Yatırım A.Ş (Menemen, Turkey) and the sun-dried dark-coloured Siyah Orak fig samples were supplied by Erbeyli Fig Research Institute (Aydın, Turkey). All chemicals used in the study were analytical grade unless otherwise mentioned and purchased from Sigma Aldrich Co., Ltd. (İzmir, Turkey).

3.2. Methods

3.2.1. Moisture Content Determination

Moisture content of fig samples were determined according AOAC official method 934.06 (AOAC, 1970). First, the empty petri dish and lid was incubated at 70 ^{o}C for 2 hours and cooled to room temperature in a desiccator. The weight of the petri dish and lid was noted. Into the dried dish 10 grams of each sample were placed and the initial weight of them was noted as W_i. They were dried at 70 ^{o}C for 6 hours under pressure ≤ 100 mmHg (13.3 kPa). Following 6 hours, samples were weighted every 2 hours until the difference in moisture content was lower than 0.2%. Then, dried samples were collected

and cooled in a desiccator to room temperature and weighted. The final weight of the samples was noted as W_f and the moisture content was calculated following equation 1.

$$Moisture\ Content\ (\%) = 100x\left(\frac{Wi - Wf}{Wi}\right) \quad (1)$$

3.2.2. Pectin Extraction

Pectin extraction from regular and coloured fig samples was conducted by hot acidic extraction method introduced by (Çavdaroğlu, Farris, and Yemenicioğlu 2020). As acidic solution 6% (w/v) citric acid (CA) solution is used. The fig samples were divided into smaller pieces, mixed with 1:3 (w/v) citric acid solution and blended using Waring blender (31BL91, Torrington, USA). The slurry obtained was heated in a hot plate with constant stirring at 95 ^{o}C. After 1 hour of heating the slurry was brough to room temperature and centrifuged at 5000 g for 20 min at 4 ^{o}C. The supernatant was filtered through cheese cloth and collected. The remaining pellet was mixed with 1:1 (w/v) CA solution, second extraction was carried out at 95 ^{o}C for 1 hour with constant stirring to extract remaining pectin. Again, the supernatant was collected and all the supernatants were combined. Into the combined supernatant solution 1:2 (v/v) 96% ethanol was added and mixed for 30 minutes. The solution then stored at 4 ^{o}C for 18 hours for pectin precipitation. Then, the solution was centrifuged at 9600 g for 10 min at 4 ^{o}C and pellet was separated. The pellet was washed by mixing with 1:1 (w/v) 96% ethanol and centrifuged at 5000 g for 10 min at 4 ^{o}C. The collected pellet was washed again with the same steps. After final centrifugation the purified pectin was collected and dried at 40 ^{o}C for 16-18 hours. The obtained dried pectin was freeze dried to decrease the moisture content below 5% using Labconco lyophilizator (Labconco, Freezone, 6L, Kansas City, USA). For each fig sample, extraction was conducted in large scale and the obtained dried regular fig pectin (FPECn) and coloured fig pectin (FPECc) were grinded to form dried pectin powder. The extraction yield was given as percentages and calculated with equation 2. Further analyses were conducted using CPEC, FPECn and FPECc powder.

$$Extraction\ yield\ (\%) = 100x\left(\frac{weight\ of\ dried\ pectin\ (g)}{weight\ of\ dried\ sample\ (g)}\right) \quad (2)$$

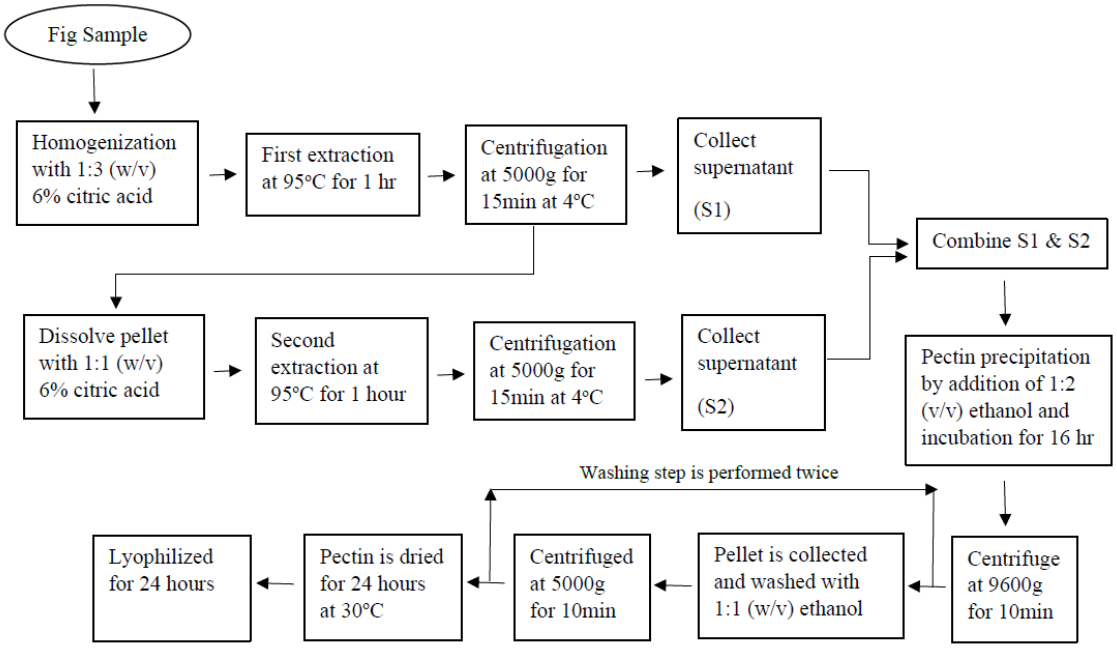

Figure 3.1. The process flow chart for pectin extraction from fig fruits

3.2.3. Molecular Characterization of Pectin and Phenolic Extracts

3.2.3.1. Degree of Esterification

Esterification degree of pectin samples were determined following the titrimetric method introduced by (Singthong et al., 2004). First 50 mg pectin powder was wetted with 0.2 mL of 96% ethanol and 10 mL dH_2O at 50 °C was added. The mixture was stirred until completely dissolved. 5 drops of phenolphthalein were added as indicator and titrated with 0.25 M NaOH solution until the turn point. NaOH volume used upon the turning point was noted as 'V_i'. Then, 5 mL of 0.1 M NaOH solution was added and solutions were stirred for 30 minutes. To neutralize the NaOH solution, after 30 minutes 5 mL of 0.1 M HCl solution was added and the mixture was stirred until the pink colour fades. Finally, the neutralized solutions were titrated with 0.25 M NaOH solution and the volume spent for colour change was noted as 'V_f'. Using equation (3), the degree of esterification was determined, and the results were expressed as percentage (%).

$$\%DE = 100\left(\frac{V_f}{V_i + V_f}\right) \quad (3)$$

3.2.3.2. Galacturonic Acid Content

Galacturonic acid content was determined by meta-hydroxyphenyl method using D-galacturonic acid as standard (Cemeroğlu 2010). In this method, distilled water at 50 ºC was added into 50 mg dried pectin powder and mixed until fully dissolved. Into glass tubes 300 µL pectin solution was poured and vortexed for 5 seconds after 125 mM sodium tetraborate solution prepared with concentrated sulphuric acid (1.8 mL) was added. The tubes were incubated in water bath at 100 ºC. After 10 minutes of incubation the tubes were cooled to room temperature with ice bath and quickly 0.15% m-hydroxyphenyl solution (30 µL) prepared with 10% (w/v) NaOH was added. Solution was vortexed and incubated at room temperature for 5 minutes. The absorbance of samples was determined at 520 nm. Standard curve was prepared by using various concentrations (0-100 µg/mL) of standard galacturonic acid solutions and the results were expressed as percentages (%).

3.2.3.3. Protein Content

Total soluble protein content of pectin samples and phenolic extracts were obtained by employing the method developed by (Bradford 1976). Initially, Bradford reagent was prepared by dissolving 100 mg of Coomassie Brilliant Blue G-250 in 50 ml 96% ethanol and 100 ml 85% phosphoric acid in a 1 L volumetric flask. The prepared solution was then completed to 1 L with deionized water, this solution was stored at 4 ºC for further analysis. For the measurement, 50 µl of sample solution was placed into glass tubes and 2500 µl of Bradford reagent was added into each and vortexed. The absorbance of each sample was measured at 595 nm after 1 hour incubation at room temperature in the dark. The standard curve was prepared with BSA and for blank samples dH_2O was used. The results were expressed as mg BSA/g pectin.

3.2.3.4. Total Phenolic Content

Total phenolic content of pectin samples and phenolic extracts were examined by applying the methodology first introduced by (Singleton and Rossi 1965). Prior to each measurement 10% (v/v) Folin-Ciocalteu's reagent and 7.5% (w/v) $NHCO_3$ solution were prepared freshly. For each measurement, 200 µl of appropriately diluted sample solution was placed into glass test tubes and 800 µl of sodium carbonate solution was added and vortexed. The mixture was incubated in dark for 5 minutes then 1 ml of Folin-Ciocalteu's reagent was added. The solution was vortexed and incubated in the dark for 1 hour at room temperature. The absorbance of mixtures was measured at 760 nm. As the standard catechin was used and the total phenolic content of each sample was expressed as catechin equivalent (for pectin samples and phenolic extracts; mg CAT/g sample).

3.2.3.5. Evaluation of Sugar Content and Sugar Molar Ratios

The sugar compositions of the pectin samples were measured spectrophotometrically using enzymatic kits namely K-ARGA kit for galactose and arabinose content and K-RHAMNOSE kit for rhamnose content according to Çavdaroğlu and Yemenicioğlu (2022). Prior to analysis pectin samples were hydrolyzed by addition of 5 ml 1.3 M HCl solution and incubation at 100 ^{o}C water bath for 1 hour. Samples were cooled to room temperature in an ice bath and neutralized (pH=7) with 1.3M NaOH solution and the volume was completed to 20 ml with dH_2O. Prior to measurement the solutions were filtered through 45 µm syringe filter and diluted with dH_2O. Sugar content analysis were conducted by following the procedures of each kit's manual. The measurements were conducted in duplicates. The homogalacturonan content (HG) and rhamnogalacturonan-I content (RG-I) was calculated using equations 4 & 5.

$$HG\ (\%) = GalU(\%) - Rha(\%) \quad (4)$$

$$RG - I(\%) = [GalU(\%) - HG(\%)] + Rha(\%) + Gal(\%) + Ara(\%) \quad (5)$$

3.2.4. Preparation of Emulsions and Determination of Emulsification Capacity and Emulsion Stability

The method introduced by Raji et al. (2017) was used with slight changes for the determination of emulsion capacity. Initially, emulsions were prepared with 0.25, 0.5, 1 & 2% (w/v) pectin concentrations. Various phenolic concentrations were also prepared; 0.1, 0.5 & 1% (w/v) concentrations were prepared for GTE & GSE and 1 & 2% (w/v) concentrations were used for CAT samples. The prepared pectin-phenolic solutions were mixed with 1:1 (v/v) olive oil and homogenized at 20000 rpm for 6 minutes using a homogenizer (Heidolph, silent crusher M, Schabach, German, rotor ϕ = 6.6 mm). The emulsion samples were placed into graduated cylinders and incubated for 14 days at 25 ^{o}C. The phase separation was read as volume from the graduated cylinders at 30 minutes, 6 hours, 1 day, 3 days, 5 days, 7 days, and 14 days. The emulsion stability (ES) was calculated using Equation 6, where ELV is emulsifier layer volume and E_v is total volume.

$$\%ES = 100\left(\frac{ELV}{Ev}\right) \quad (6)$$

3.2.5. Characterization of Emulsions

3.2.5.1. Determination of Particle Size

The droplet size measurements were performed at days 1, 7 & 14 during storage to determine the stability of the emulsions using a dynamic light scattering instrument (ZetaSizer - NanoPlus, Micromeritics Instrument Corporation, GA, USA). The refractive index for water was 1.333 and for olive oil 1.472. The results of droplet size expressed as volume-weighted mean particle diameter (d_{43}) were given as µm.

3.2.5.2. Determination of Zeta Potential

The zeta potential of the pectin emulsions was measured by a dynamic light scattering instrument (ZetaSizer - NanoPlus, Micromeritics Instrument Corporation, GA, USA). The measurements were conducted at set time intervals (days 1, 7 & 14) during storage of the emulsion samples at 25 ^{o}C. Emulsion samples were diluted to 1:100 (v/v) with dH_2O to prevent multiple scattering effects and obtain measurable readings. The results were expressed as mV.

3.2.5.3. Determination of Emulsion Viscosity

The viscosity of emulsions was measured according to the method proposed by (Monsoor 2005) with slight modifications. Prior to viscosity measurements the samples were allowed to stabilize for 5 minutes. Following stabilization, viscosity measurements were obtained using a viscometer (DIN 5309, Rotor SV, Haake VT550 Thermo Electron Corp., Karlsruhe, Germany) at room temperature. The results were expressed as Pa.s at 10 s^{-1}.

3.2.5.4. Evaluation of Antioxidant Oxidant Activity During Storage

ABTS radical scavenging activity method was used to determine the antioxidant activity of samples as introduced by Re et al. (1999) with slight modifications. Samples were prepared by diluting the emulsion with dH_2O in various concentrations (1:10-1:75 (v/v)). Following mixing, they were centrifuged at 10000xg for 10 min at 4 ^{o}C and the obtained clear serum was used for the experiment. Initially, 75 mM phosphate buffer solution (PBS) containing 150 mM NaCl was prepared at pH 7.4 and 2.45 mM/L potassium persulfate was prepared with PBS. Using the potassium persulfate solution, 7 mmol/L ABTS stock solution was prepared and incubated for 16 hours in the dark with constant mixing. Prior to each measurement, $ABTS^{\bullet+}$ stock solution was diluted with PBS to yield 700±20 absorbance at 734 nm. For the measurement, into 30 µl of diluted sample solution 2 mL of $ABTS^{\bullet+}$ solution was added and mixed instantly. The readings were obtained kinetically for 15 minutes. For control reading PBS was used instead of sample solution.

The results were first determined in case of (%) inhibition and expressed as Trolox equivalent (mmol Trolox/ml emulsion).

$$\%Inhibition = \frac{(Initial\ absorbance) - (Final\ absorbance)}{(Initial\ absorbance)} x100 \qquad (7)$$

3.2.5.5. Evaluation of Total Phenolic Content During Storage

The phenolic content of emulsions was measured by following the same methodology given in section 3.2.3.4. The analysis was conducted with samples taken at days 1, 3, 5, 7 and 14. For the measurement, the emulsions were first mixed 1:10 (v/v) with distilled water and vortexed. The emulsions were then disturbed by centrifugation at 10000xg for 10 min at 4 ^{o}C. The clear aqueous phase at the bottom of centrifuge tube was then further diluted and quantified for its phenolic content. The results were expressed as mg CAT/ml emulsion.

3.2.5.6. Statistical Analysis

The data obtained from the analyses in triplicates and expressed as the mean value ± standard deviation. The statistical analysis was performed using Minitab (Version 18.1., Minitab Inc., United States). Statistically significance analysis was obtained using one-way ANOVA with Fisher's post hoc test with 95% confidence level ($P < 0.05$).

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Pectin Extraction and Characterization

The pectin extraction yield (% of dry weight) is the percentage of pectin found in fig fruits. The pectin extraction yields were found 7.49% and 8% for dark-coloured Siyah Orak and Sarilop figs, respectively. This result suggested slightly higher pectin content for the traditional Sarılop cultivar than the newly breed coloured fig cultivar. Previous studies in the literature conducted with similar or different hot citric acid extraction conditions, the pectin extraction yield from waste sun-dried fig puree was found between 6.7 and 9.12% (Çavdaroğlu, Farris, and Yemenicioğlu 2020) while pectin extraction yield from waste stalks of processed sun-dried figs was found between 6.30 and 12.40% (Çavdaroğlu and Yemenicioğlu 2022). Thus, it is clear that the pectin extraction yield in the current thesis was between the limits reported in the literature.

The characterization of the extracted fig pectins, Sarılop (FPECn) and Siyah Orak fig pectins (FPECc), in comparison with CPEC was conducted by determination of their galacturonic acid content (GA), sugar concentrations (arabinose, galactose, rhamnose), homogalacturonan (HG) and rhamnogalacturonan-I (RG-I) contents, degree of esterification (DE), protein content, total phenolic content and antioxidant activity (Table 4.1).

The galacturonic acid content for CPEC (94%) was expectedly higher than those of FPECn (33%) and FPECc (35%) that showed quite similar GA content. The determination of galactose, arabinose and rhamnose content was also conducted for all pectins. There were no significant differences among the rhamnose contents of three samples. However, galactose and arabinose contents of FPECc were noticeably lower than those of both CPEC and FPECn. The HG content that shows the homogeneity of pectins and RG-I contents that shows the amount of hairy region in pectins were also calculated form composition of major sugars (Zheng et al. 2020).

The RG-I of FPECn sample was considerably higher than those of both CPEC and FPECn while the HG of FPECn was significantly lower than those of the other two pectins (Table 4.1). The amounts of both HG and RG-I moieties of pectin effect the emulsification ability hence the HG/RG-I ratio plays a key role for pectin's ability to form stabile emulsions (Ngouémazong et al. 2015). The highest HG/RG-I ratio was observed for CPEC and FPECc while FPECn showed the lowest HG/RG-I ratio.

The DE of pectin samples was 68.10, 19.40 and 29.6 for CPEC, FPECn and FPECc, respectively. The esterification degree of all three pectins were significantly different than each other with CPEC having the highest DE followed by FPECc and FPECn. Recently, DE of substandard sun-dried fig pectin was reported as 38% by Çavdaroğlu and Yemenicioğlu (2022). In another study pectin was obtained through several methods from peels of fresh figs and its DE was found between 32.41-39.42% (Gharibzahedi, Smith, and Guo 2019). Thus, it seemed that the DE values determined for FPECn and FPECc were significantly and slightly lower than those reported in the literature, respectively. However, it is hard to understand the exact factors causing differences in DE since differences in pectin methylesterase enzyme activity fig varieties, drying conditions mediated by seasonal variations in climate, and extraction conditions might show significant variations in DE of fig pectins.

The results of protein content determination showed that FPECc (10.16%) had the highest protein content followed with FPECn (7.37%) and CPEC (1.86%). These results correlated well with those in the literature since protein content of sun-dried fig pectin was reported as 11% by (Çavdaroğlu and Yemenicioğlu 2022). The protein content of pectins is commonly associated with their ability to form stable emulsification since some proteins show excellent emulsifying activity offing to their conformation and amphiphilic nature (Leroux et al. 2003).

The total phenolic content and the antioxidant activity are expectedly parallel with each other since the antioxidative activity is mainly attributed to phenolic content. The FPECc showed the highest phenolic content and antioxidant activity, followed by FPECc and CPEC. This result was expected since sun-dried figs are rich in polyphenols and figs lost their tissue integrity during sun-drying allowing pectin to contact with polyphenols.

Table 4.1. Various characteristics of citrus and fig pectins

		CPEC	FPECn	FPECc
Yield*	%	-	8	7.49
Degree of esterification*	%	68.10±2.12[a]	19.40±1.14[c]	29.58±0.59[b]
Galacturonic acid content*	mg GA/g pectin	80.09±3.14[a]	30.63±0.28[b]	28.41±0.65[b]
Protein content*	g BSA/100g pectin	1.86±0.25[c]	7.37±0.37[b]	10.16±0.98[a]
Total phenolic content*	g CAT/100g pectin	0.55±0.04[c]	2.17±0.08[a]	1.93±0.01[b]
Antioxidant activity*	mmol Trolox /100g pectin	16.82±0.38[c]	29.56±1.67[a]	21.86±1.30[b]
Galactose content*	g/100g pectin	6.24±0.69[a]	5.26±1.36[a]	2.04±0.71[b]
Arabinose content*	g/100g pectin	7.65±0.32[a]	7.62±3.66[a]	3.30±0.65[b]
Rhamnose content*	g/100g pectin	1.45±0.50[a]	1.05±0.42[a]	0.76±0.36[a]
HG*	g/100g pectin	81.71±0.58[a]	70.73±1.89[b]	79.22±2.29[a]
RG-I*	g/100g pectin	18.29±0.58[b]	29.27±1.89[a]	20.78±2.29[b]
HG/RG-I*	g/100g pectin	4.47±0.18[a]	2.42±0.22[b]	3.84±0.53[a]

*Values are given as mean ± standard deviation. Values shown in each row indicated by different letters are significantly different ($P < 0.05$).

4.2. Effect of Pectin Concentration on Emulsion Stability

Effects of various pectin concentrations between 0.125 and 1% (w/v) on olive oil-in-water (O/W) emulsion stability have been investigated for all three pectin types. The emulsions cannot be formed without pectin. Moreover, the addition of 0.5% GTE or GSE without the presence of pectin cannot form stabile emulsions and destabilized rapidly. The changes in stability of CPEC emulsions with varying pectin concentration have been shown in Fig. 4.1. During 14-days storage at room temperature, 1% CPEC was able to form quite stable emulsions that showed no significant emulsion loss for 5 days. In contrast, emulsions prepared with 0.125, 0.25 and 0.5% CPEC showed significant losses (≥ 36%) in their stabilities within day 1. The most rapid destabilization was observed at CPEC concentration of 0.125% followed decreasing order by pectin concentrations at 0.25 and 0.5%.

The results of emulsification capacity for FPECn and FPECc are also presented in Figures 4.2. and 4.3., respectively. The emulsions prepared with FPECn at 0.125 and 0.25% showed poor emulsion stability, but FPECn at 0.5 and 1% showed moderate and good emulsion stability, respectively.

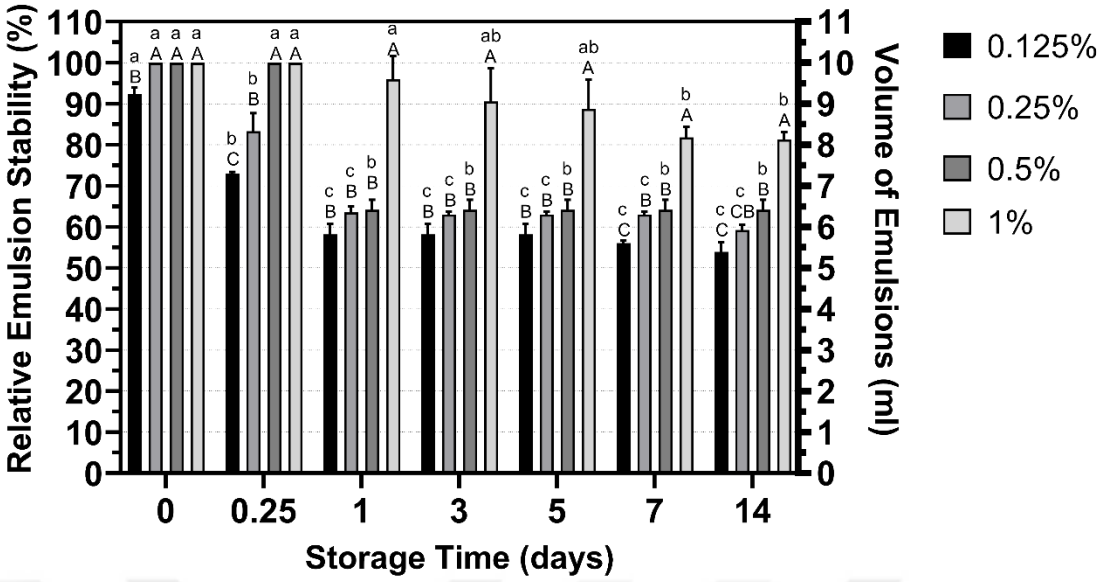

Figure 4.1. The effect of CPEC concentration (0.125-1%) on emulsion stability during 14-days storage at room temperature (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

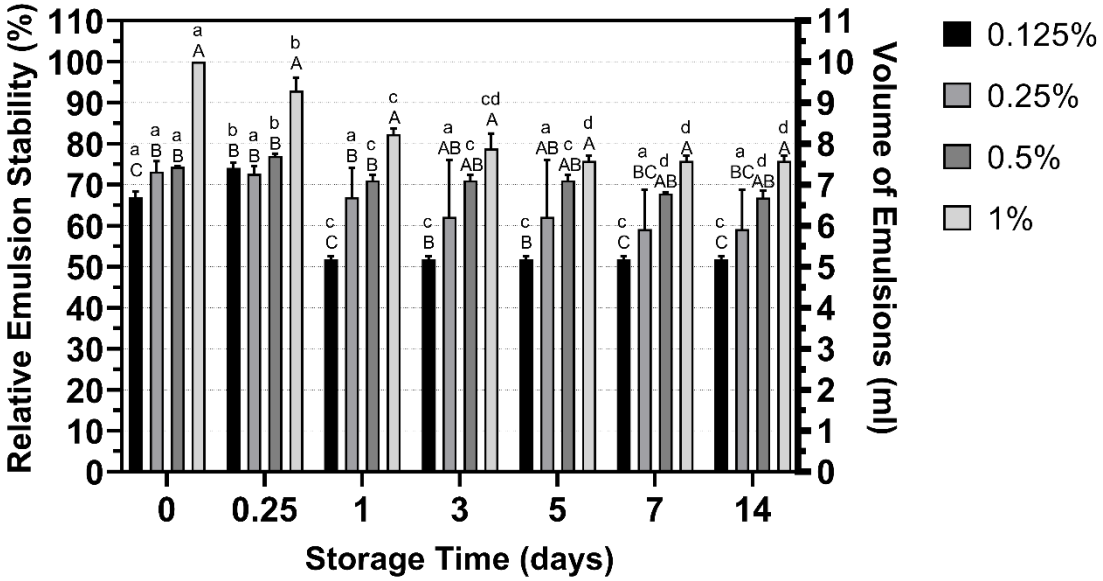

Figure 4.2. The effect of FPECn concentration (0.125-1%) on emulsion stability during 14-days storage at room temperature (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

The emulsion stability results for FPECc showed that this pectin showed slightly higher emulsion stability than FPECn at 0.125% pectin concentration. The FPECc and FPECn showed comparable emulsion stabilities with each other at 0.25 and 0.5% pectin concentrations, but FPECc at 1% performed slightly better than FPECn at 1%. It is also important to note that the emulsion stability performance of FPECc is close to that of CPEC. However, the differences among the overall emulsion stability performances of CPEC, FPECn and FPECc were not considerably different from each other considering the retention of almost 81, 75, and 80% of their initial emulsion capacity at 1% pectin concentration after 14-days storage.

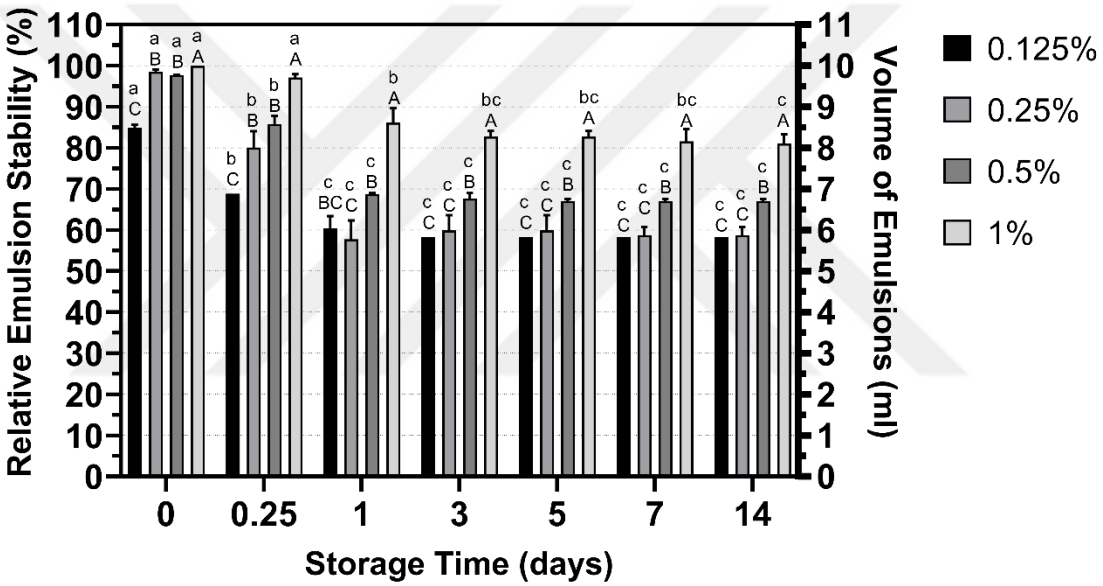

Figure 4.3. The effect of FPECc concentration (0.125-1%) on emulsion stability during 14-days storage at room temperature (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

The results obtained for all three types of pectin were in parallel to each other in case of the effect of pectin concentration on emulsion stability. On day 14 of the storage, as the concentration is increased from 0.125 to 1% emulsion stability increased 51% for CPEC, 46% for FPECn and 39% for FPECc.

In the current thesis the high oil to water ratio (1:1) was preferred in the emulsion formation to simulate foodstuff with high oil content such as salad dressing and meal sauces. In the literature, the emulsion stability of pectin extracted by several different

methods from fresh fig peel were studied by Gharibzahedi, Smith, and Guo (2019). These researchers reported that the fresh fig peel pectin emulsions maintained 83 to 95% of their stability by 1-day storage at 24 ^{o}C. However, these findings were not comparable with results of current study since Gharibzahedi, Smith, and Guo (2019) used 5% corn oil in the emulsions and applied sonication to form their emulsions. It is also important to note that the storage period applied by these workers was too short to evaluate stability of typical food emulsions.

4.3. Effect of Green Tea and Grape Seed Phenolic Extracts on Emulsion Stability

In this study, the stability of emulsions in the presence of polyphenols was studied at pectin concentration of 0.5% that is equal to half the pectin concentration (1%) necessary to obtain a stable olive oil-in-water emulsion. This strategy allowed to determine the emulsion stabilizing effects of different polyphenols. Effects of green tea (GTE) and grape seed (GSE) extract concentrations at 0.05, 0.25 and 0.5% (w/v) on stability of olive oil-in-water emulsions prepared with 0.5% (w/v) pectin are seen in Figures 4.4 to 4.9.

The control olive oil-in-water emulsion with CPEC at 0.5% without the additional polyphenols lost 36% of its stability in 1 day. In contrast, CPEC emulsion stability in the presence of GSE or GTE was improved significantly (Figure 4.4). For example, in the presence of GTE both at 0.05 and 0.25% the loss in CPEC emulsion stability was 23% within 7 days of storage. Interestingly, the increase of GTE to 0.5% did not cause further increase in CPEC emulsion stability (32% loss in stability within 1 week). This shows that the excess GTE in the emulsion causes unfavourable changes in the stability. A similar finding was also obtained for whey protein-tea polyphenol emulsions in a study conducted by Tian et al., (2022). It was reported that the increase of tea polyphenol content from 0.01 to 0.04% decreased the stability of emulsions prepared with Tween 20. It seemed that the excessive GTE caused a slight increase in coalescence of emulsion droplets.

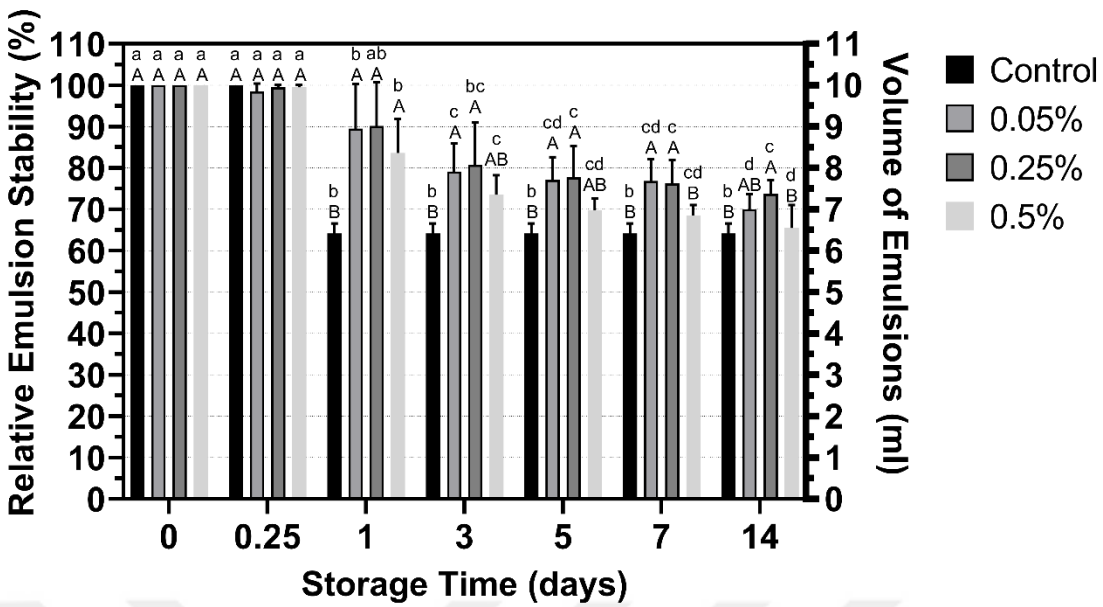

Figure 4.4. The effect of GTE concentration (0.05-0.5%) on 0.5% CPEC emulsion stability during 14-days storage at room temperature (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

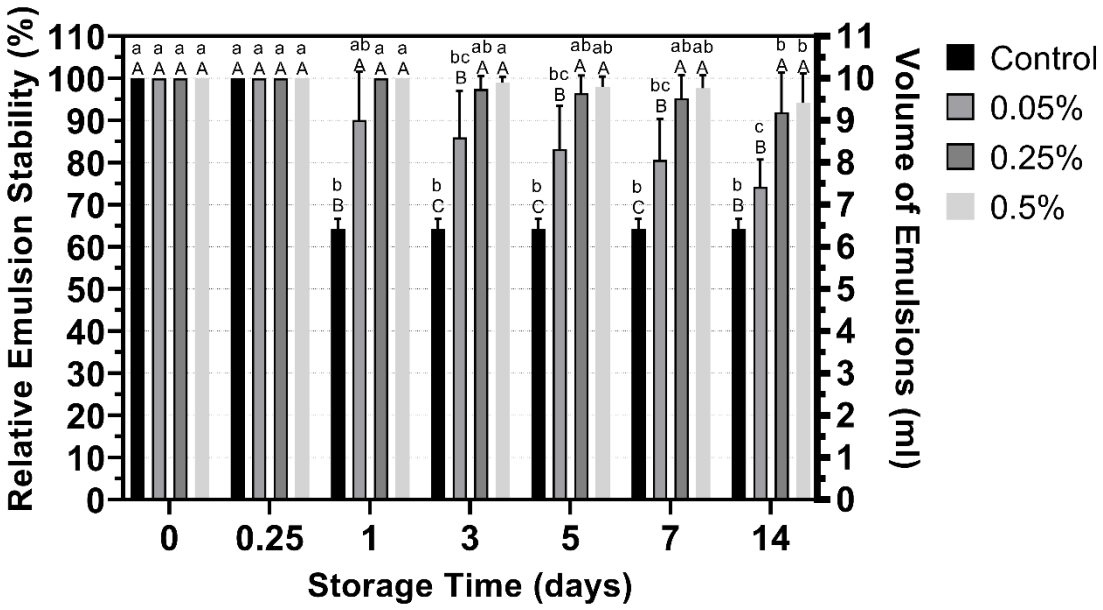

Figure 4.5. The effect of GSE concentration (0.05-0.5%) on 0.5% CPEC emulsion stability during 14-days storage at room temperature (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

The most stable CPEC emulsions were obtained in the presence of GSE (Fig. 4.5). The presence of GSE at 0.25 and 0.5% resulted with 92 and 94% emulsion stability at the end of 14 days, respectively. Thus, it is clear that the CPEC emulsion stabilizing effect of GSE is considerably higher than that of GTE.

The effect of GTE and GSE on FPECn emulsion stability are seen in Figure 4.6 and 4.7, respectively. The control FPECn emulsion without polyphenols lost 26% of its stability at day 0, but the presence of GTE at different concentrations increased the day 0 emulsification capacity of FPECn. However, the stability of FPECn emulsions gained by GTE was short-lived and lost considerably within 1 day. The FPECn emulsions with 0.05% GTE show the highest stability during storage, but GTE at higher concentrations did not cause an improvement in emulsion stability.

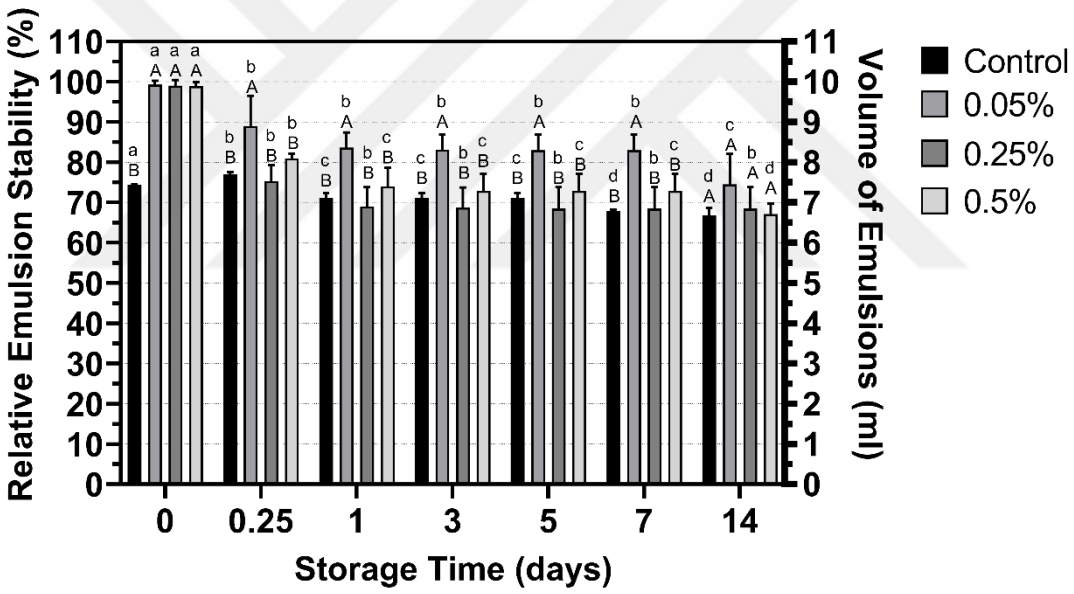

Figure 4.6. The effect of GTE concentration (0.05-0.5%) on 0.5% FPECn emulsion stability during 14-days storage at room temperature (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

Similar to the effect of GTE, GSE also caused a significant increase in the FPECn emulsification capacity at day 0. However, GSE at 0.05, 0.25 and 0.5% also showed no positive effects on FPECn emulsion stability during storage.

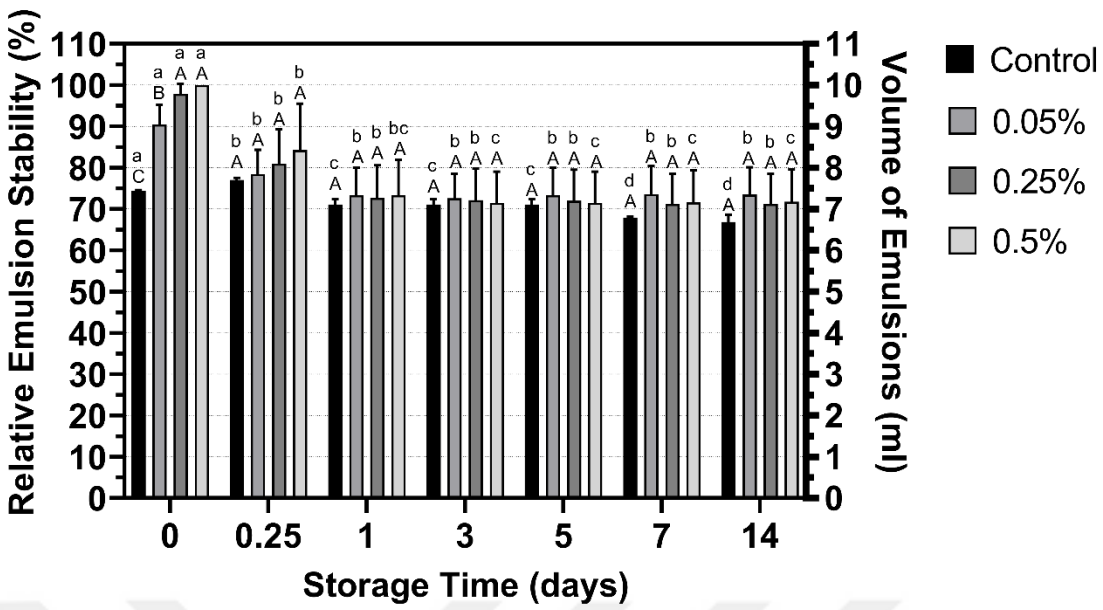

Figure 4.7. The effect of GSE concentration (0.05-0.5%) on 0.5% FPECn emulsion stability during 14-days storage at room temperature (Different letters indicate significantly different values at P < 0.05. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

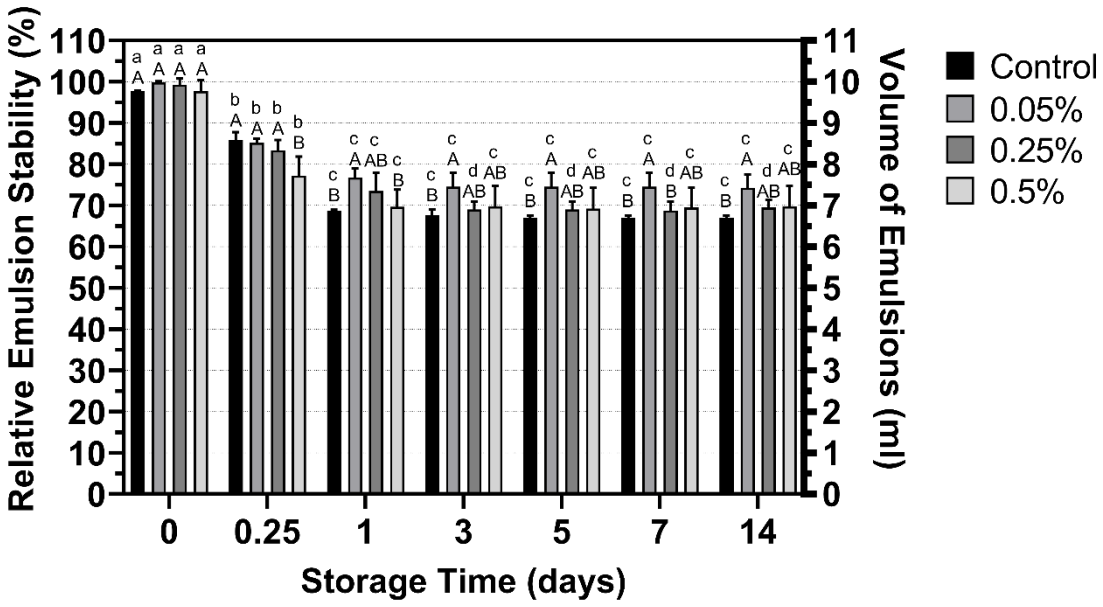

Figure 4.8. The effect of GTE concentration (0.05-0.5%) on 0.5% FPECc emulsion stability during 14-days storage at room temperature (Different letters indicate significantly different values at P < 0.05. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

The results for FPECc emulsion stability tests in the presence of GTE and GSE are also shown in Figure 4.8 and 4.9, respectively. The FPECc control sample without polyphenols showed a better emulsification capacity than FPECn at day 0. In the presence of 0.05% GTE, the stability of FPECc emulsion improved slightly, but GTE at 0.25 and 0.5% did not make a significant contribution in FPECc emulsion stability.

The FPECc emulsion stabilizing effect of GSE was found significantly higher than that of GTE. However, the emulsion stabilities in the presence of 0.05, 0.25 and 0.5% GSE decreased significantly in day 0.25 and 1 (Figure 4.9). FPECc emulsions with 0.5% GSE showed significantly higher stability at day 14 with only 24% loss of their stability. It is important to note that positive contribution of GSE in FPECc emulsion stability was much less than that in CPEC emulsions.

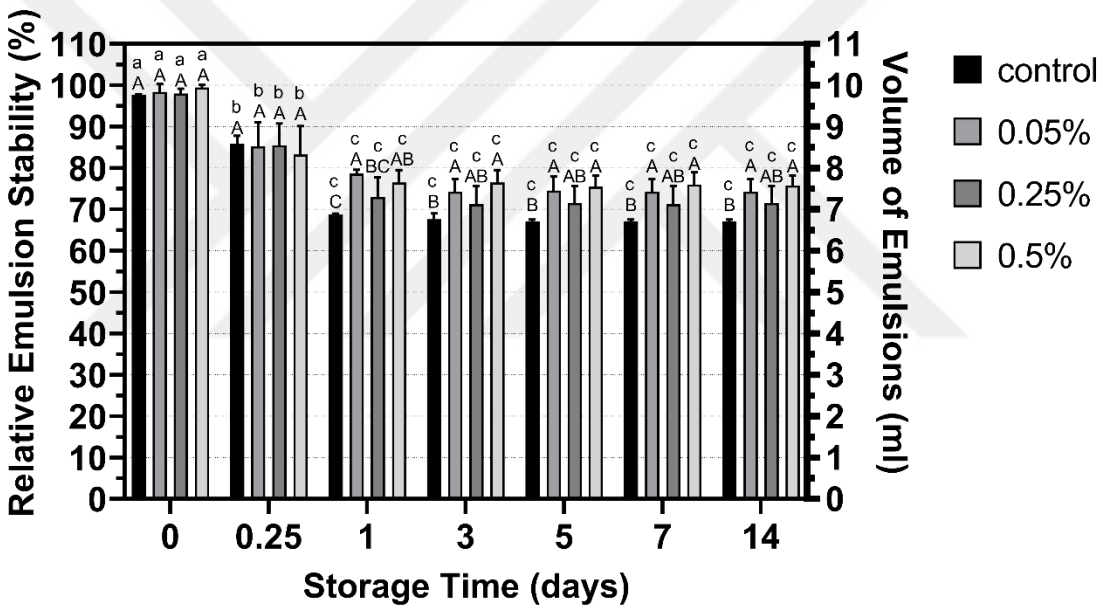

Figure 4.9. The effect of GSE concentration (0.05-0.5%) on 0.5% FPECc emulsion stability during 14-days storage at room temperature (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

The significantly higher stability of GSE than GTE added CPEC and FPECc emulsions could be in part due to differences between the total polyphenol contents of these extracts. The total polyphenol content of GSE (87 mg CAT/100g of GSE) was almost 2-fold higher than that of GTE (41 mg CAT/100g of GTE). However, the higher emulsion stability of GSE at 0.25% than GTE at 0.5% in CPEC emulsions suggested that the differences

between molecular properties of the polyphenols in these extracts is also a major factor affecting their emulsion stabilities. GTEs are rich mainly in monomeric catechins such as epigallocatechin gallate, epigallocatechin and epicatechin gallate (Yanagida et al. 2006) while GSEs are rich mainly in monomeric catechins (e.g., (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-o-gallate), and their oligomers (2-7 subunits) and polymers (8 to 24+ monomers) called proanthocyanidine (Kennedy, Matthews, and Waterhouse 2000).

4.4. Effect of (+)-Catechin on Emulsion Stability

A major monomeric catechin, CAT, was also evaluated for its emulsion stabilizing activity, but due to the positive contribution of CAT on all pectin emulsions this flavonoid was also tested at a higher concentration at 1% (w/v). The emulsion stability of different pectins in the presence of CAT were seen in Figure 4.10 to 4.12.

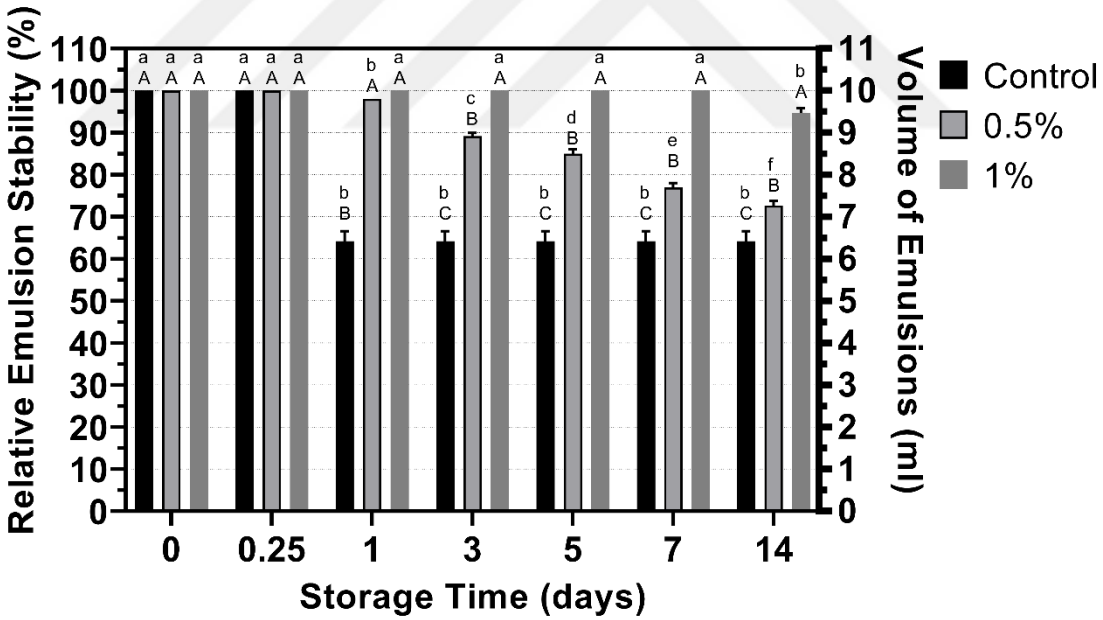

Figure 4.10. The effect of CAT concentration (0.5 & 1 %) on 0.5% CPEC emulsion stability during 14-days storage at room temperature (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

The CPEC emulsions with 0.5% CAT lost almost 27% at the end of 14 days while increase of CAT to 1% gave highly stable emulsions that showed only 5% decrease in the

stability at the end of 14 days. It should be noted that the effect of 1% CAT on CPEC emulsions is similar to the effect of 0.5% GSE at day 14 of the storage. The stability of FPECn emulsions also improved significantly with CAT. For example, FPECn emulsions prepared with 0.5 and 1% CAT maintained almost 80 and 89% of their stabilities at the end of 14 days (Figure 4.11). It appeared that the CAT at 0.5% was more effective on stabilization of FPECn than CPEC. However, CAT at 1% is more effective on emulsion stability of CPEC than that of FPECn. The slightly different responses of FPEC and CPEC against changes in CAT concentrations might be attributed to differences in their purities that interfered with CAT-pectin interactions. In fact, parallel results obtained for FPECn, and FPECc emulsions with CAT supported this hypothesis. However, the FPECc emulsions with 0.5 and 1% CAT maintained 74 and 84% of their stabilities at day 14 (Figure 4.12). Thus, it is clear that the stabilizing effect of CAT on FPECn emulsions was greater than that on FPECc. The overall results with CAT clearly showed that the CAT is the only polyphenol effective on stabilization of both citrus and fig pectin emulsions.

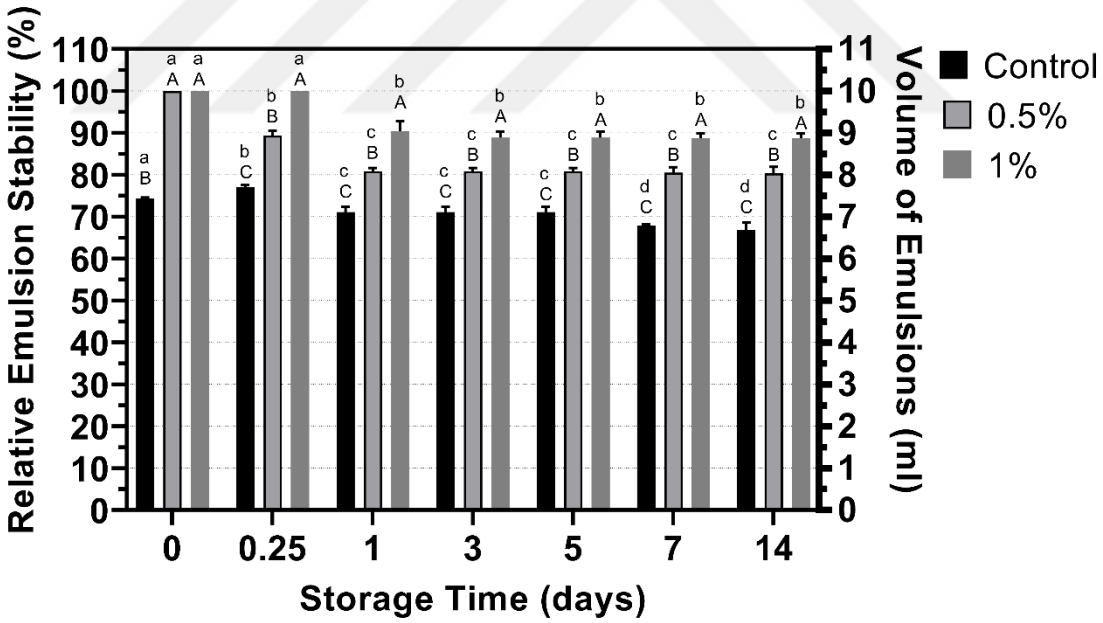

Figure 4.11. The effect of CAT concentration (0.5 & 1 %) on 0.5% FPECn emulsion stability during 14-days storage at room temperature (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

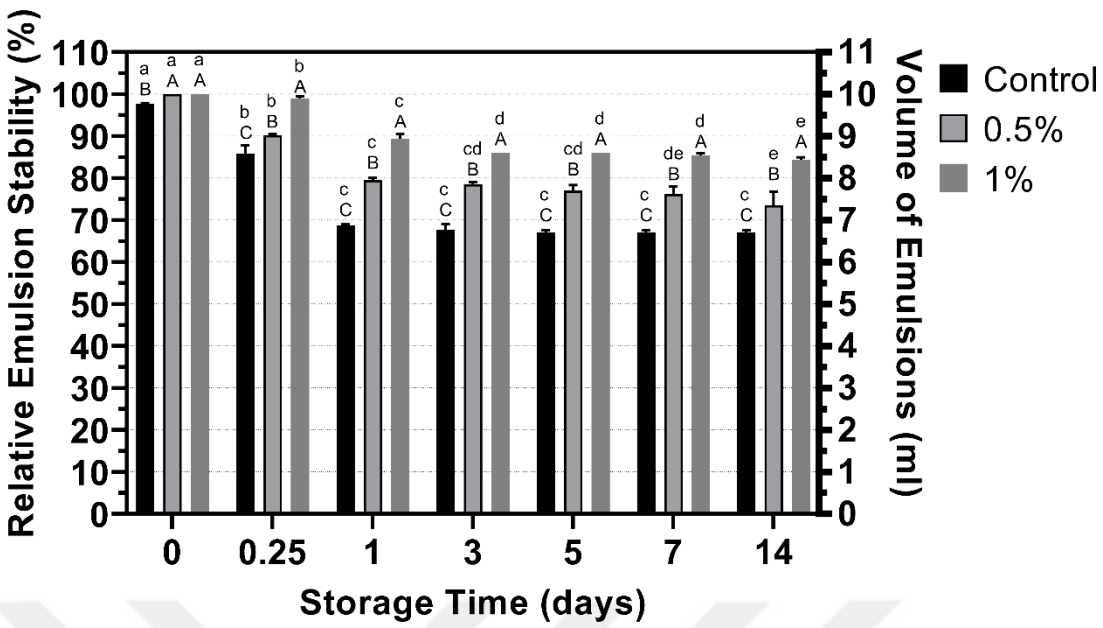

Figure 4.12. The effect of CAT concentration (0.5 & 1 %) on 0.5% FPECc emulsion stability during 14-days storage at room temperature (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

4.5. Effect of Polyphenols on Droplet Size of Emulsions

The effects of 0.5% (w/v) GSE or GTE, and 0.5 or 1% CAT on droplet sizes of CPEC emulsions during storage are presented in Figure 4.13. The results clearly showed that addition of GSE and CAT caused a dramatic reduction in initial droplet size (4.4 to 3.1-fold smaller) of CPEC emulsions. In contrast, the presence of GTE caused a more limited reduction in initial droplet size (1.4-fold smaller) of CPEC emulsions. The control CPEC emulsions and GTE, GSE or CAT added emulsions did not show significant changes or show only slight fluctuations in their emulsion droplet size during 14 days of storage.

The addition of GTE, GSE or CAT caused significant reductions in the droplet sizes of FPECn and FPECc emulsions (Figure 4.14 and 4.15). In day 1, the most significant reductions in emulsion droplet sizes occurred in the presence of 1% CAT for both FPECn and FPECc. The reductions of particle size in respect to controls for FPECn emulsions were 7.9-fold by 1% of CAT, 3.3-fold by 0.5% of CAT, 2.3-fold by 0.5% of GSE and 1.2-fold by 0.5% of GTE at day 1. The FPECc emulsions also showed somehow similar reductions in their emulsion droplet sizes; 7.2-fold by 1% of CAT, 2.3-fold by 0.5% of

CAT, 2.1-fold by 0.5% of GTE at day 1. It should be noted that the droplet sizes of emulsions prepared with FPECn and FPECc were smaller than those prepared with CPEC.

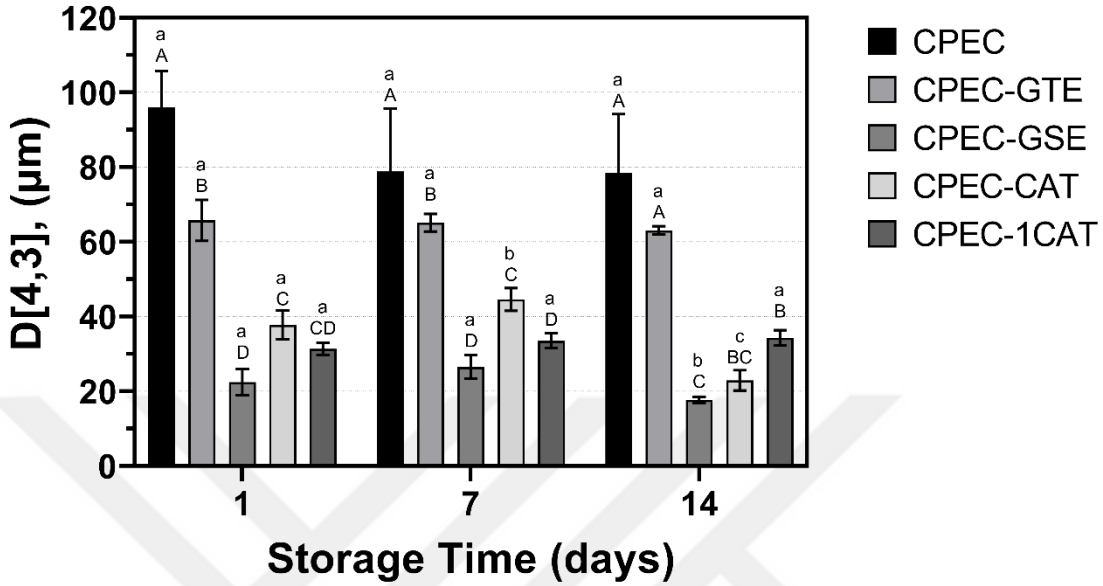

Figure 4.13. Droplet sizes of different 0.5% CPEC- (0.5-1%) polyphenol emulsions (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

The emulsion droplet sizes for control FPECn and FPECc emulsions showed a significant drop at the end of 14 days of storage. However, FPECn emulsions in the presence of 0.5% of GSE, and 0.5 or 1% of CAT; and FPECc emulsions in the presence of 0.5% of GSE or CAT did not change their emulsion droplet sizes significantly at the end of 14 days. In contrast, emulsion droplet sizes in the presence of 0.5% GTE reduced significantly after 14 days in the presence of GTE for FPECn emulsions while reductions in FPECc emulsion droplet size in the presence of GTE by storage were not significant. A significant increase in emulsion droplet size was also observed in the presence of 1% CAT for FPECc emulsions by 14-days storage, but these emulsions still showed similar droplet sizes with other polyphenol added emulsions.

The overall results of emulsion droplet size measurements clearly showed that emulsions that showed a significant increase in stability by addition of polyphenols (GSE or CAT added CPEC, and CAT added FPECn or FPECc) showed small initial emulsion droplet

size and most maintained their sizes during storage. These findings suggested that the presence of phenolic compounds such as GSE (for CPEC emulsions) and CAT (for all pectin emulsions) helps emulsion stabilization by reduction and fixation of emulsion droplet size. Although this mechanism worked particularly for stabilization of GSE and CAT added CPEC and CAT added FPECn and FPECc added emulsions, it failed for all GTE added emulsions due possibly to limited reductions in initial emulsion droplet sizes with this phenolic extract. The reduced droplet size of oil-in-water emulsions in the presence of phenolic compounds was also noted by Z. Liu et al., (2020) who achieved stabilization of emulsions prepared by citrus and apple pectins by addition of ferulic acid. However, this study reported that the reduction in droplet size caused by ferulic acid was much more pronounced in apple pectin stabilized emulsions than in citrus pectin stabilized ones. These findings suggested that the molecular properties of pectin and its ability to interact with polyphenols are major factors affecting the droplet size of pectin stabilized emulsions.

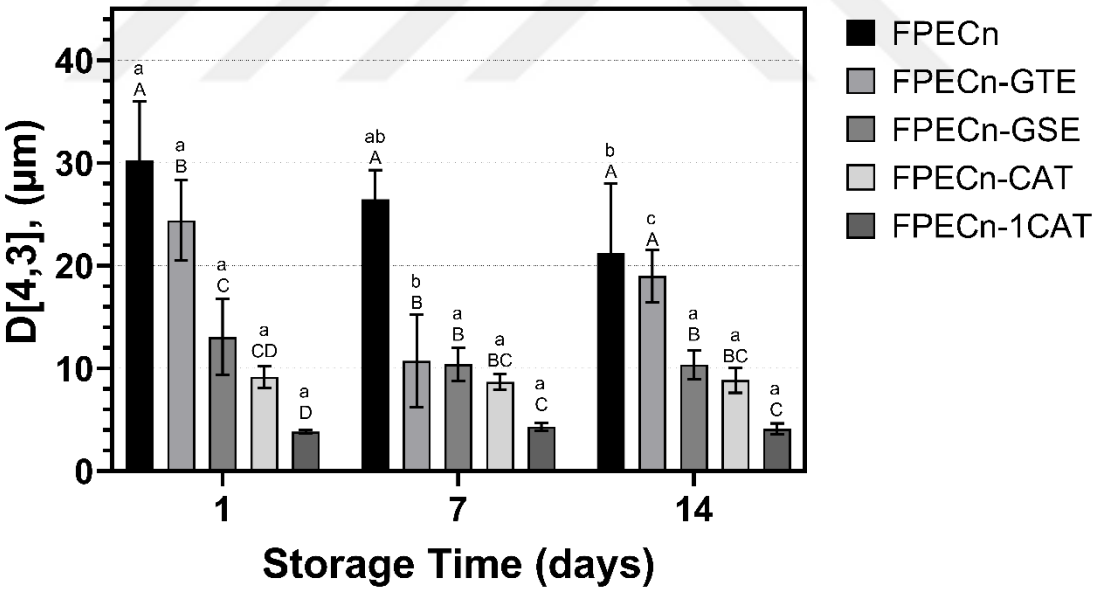

Figure 4.14. Droplet sizes of different 0.5% FPECn- (0.5-1%) polyphenol emulsions (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

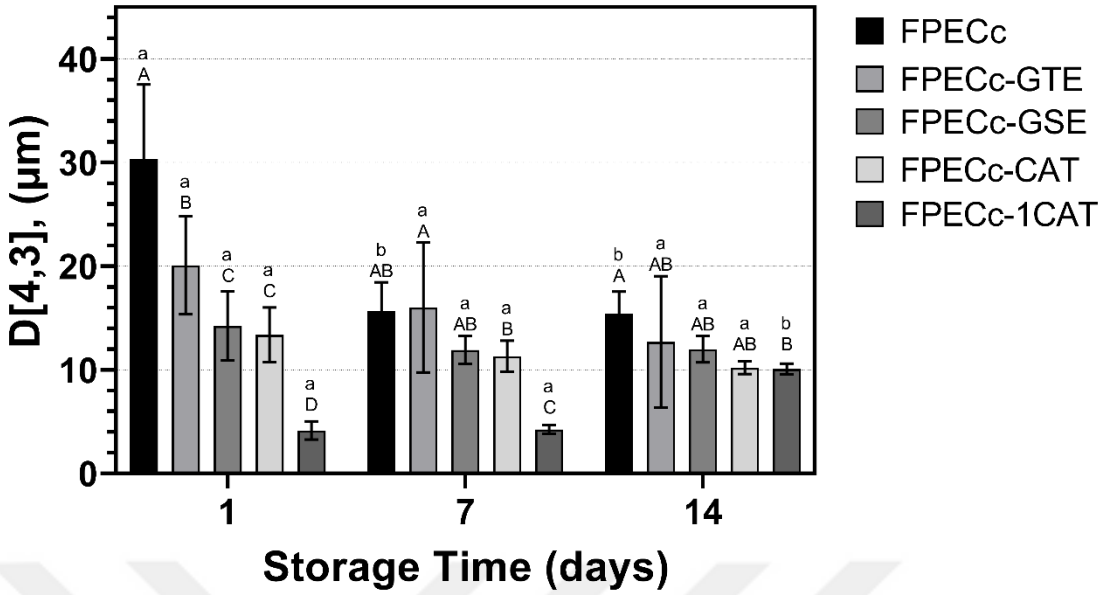

Figure 4.15. Droplet sizes of different 0.5% FPECc- (0.5-1%) polyphenol emulsions (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

4.6. Effect of Polyphenols on Zeta Potential of Emulsions

The zeta potentials of emulsions stabilized with GTE, GSE, CAT and 1% CAT (1CAT) are given in Figure 4.16 for CPEC, Figure 4.17 for FPECn and Figure 4.18 for FPECc. As expected, different emulsions prepared with CPEC yields negative zeta potential values varying between -26.91 to -29.17 mV at day 1. The addition of GSE and CAT significantly reduced the zeta potential while the others did not significantly change the initial charge intensity of emulsions (Figure 4.16).

Zeta potential values of emulsions prepared with FPECn and FPECc pectins were only examined with the addition of GTE and GSE. The FPECn emulsions showed negative zeta potential values varying between -27.11 to -27.72 mV at day 1 (Figure 4.17). The addition of GTE and GSE did not significantly affected the zeta potential during 14 days of storage.

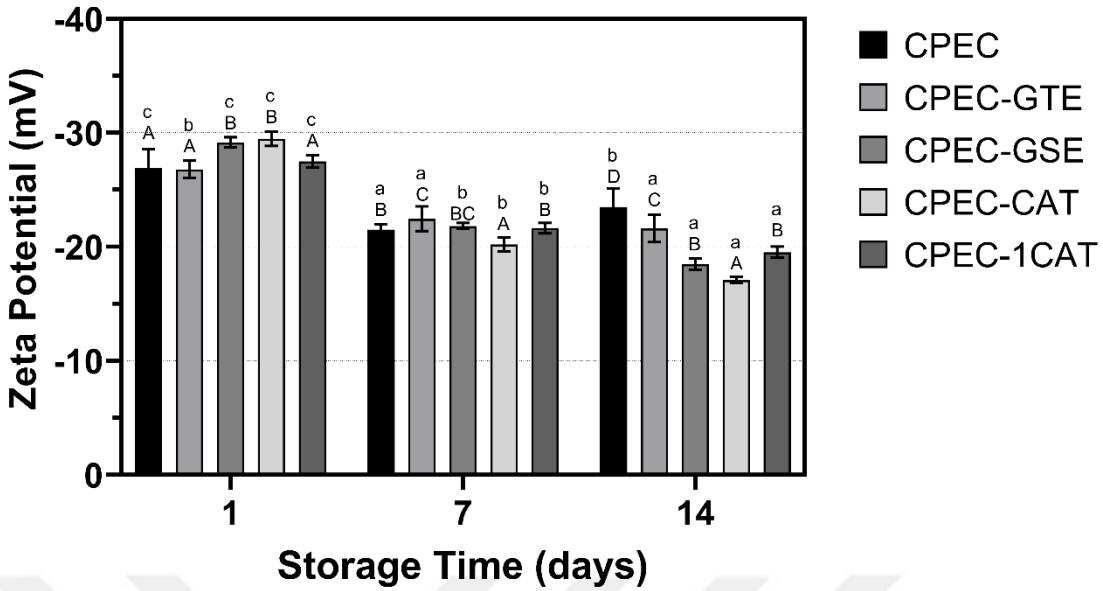

Figure 4.16. Zeta potentials of different 0.5% CPEC- (0.5-1%) polyphenol emulsions (Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

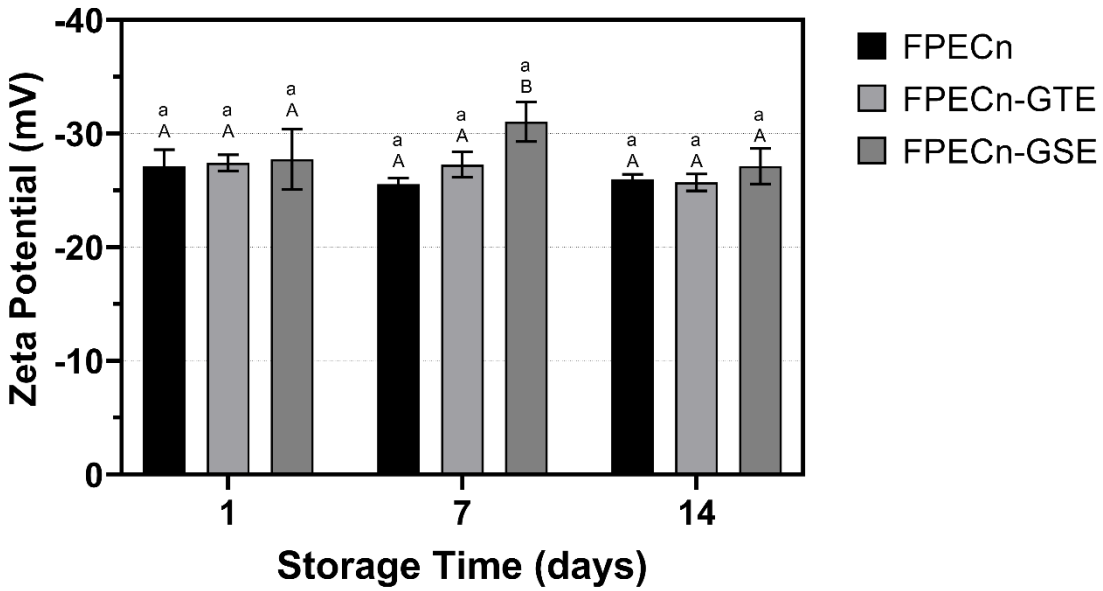

Figure 4.17. Zeta potentials of different 0.5% FPECn- (0.5-1%) polyphenol emulsions (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

The zeta potentials of FPECc emulsions varied between -25.91 to -29.32 mV at day 1 (Figure 4.18). The addition of GTE significantly decreased the zeta potential at day 1 when GSE did not show significant effect on the charge density of the sample. The zeta potential of FPECc-GSE sample increased significantly at day 7 of storage. The decrease in the zeta potential of emulsions suggests that a high energy barrier between droplets is formed which increases the electrostatic repulsion between droplets.

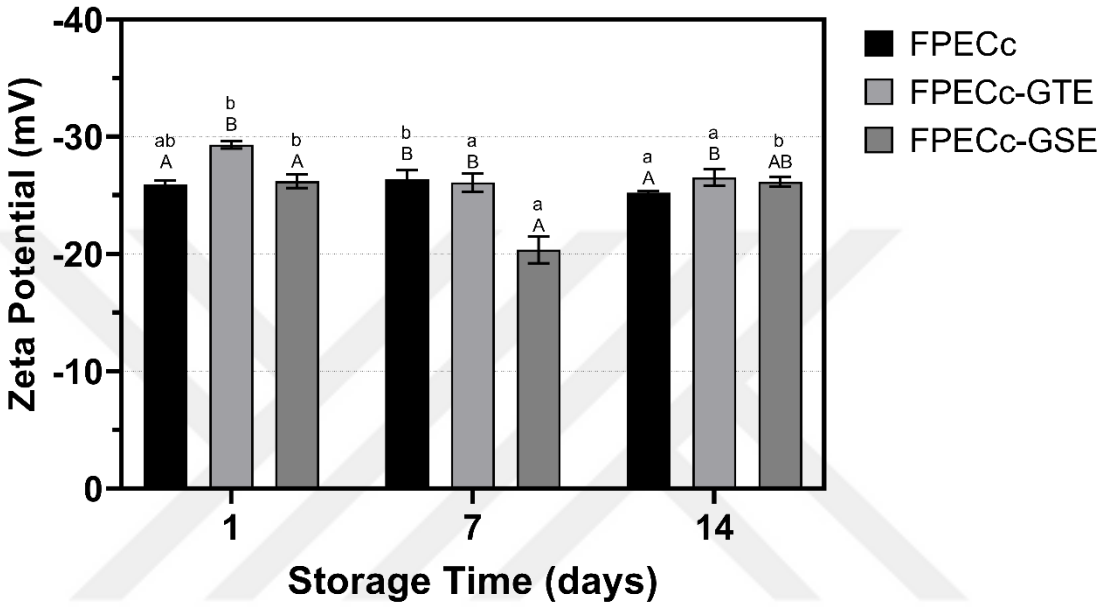

Figure 4.18. Zeta potentials of different 0.5% FPECc- (0.5-1%) polyphenol emulsions (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

Thus, the emulsion stabilizing activity of polyphenols could not be attributed to enhanced repulsion among emulsion droplets due to increased negative charges by the absorbed polyphenols at their surface. However, the storage of emulsions caused different changes in zeta potentials of emulsions. For example, GSE and CAT added emulsions showed significantly smaller negative zeta potentials than control and GTE added emulsions. This finding indicated that the masking of negative charges in pectin (carboxyl groups) occurred in GSE and GTE added emulsions during storage. This might be related to binding of GSE and CAT on hydrophobic sites (e.g., methyl ester groups) of homogalacturonan (HG) chains of pectin that resulted with reduced linearity (folding) and masking of carboxyl groups. Also, it could be related to thicker or denser layer

formation (causes masking of carboxyl groups) of polyphenol-pectin complexes around lipid droplets. It is also evident that the GTE showed less interaction with pectin or caused alternative less compact arrangements of pectin molecules with limited masking of their free carboxyl groups.

4.7. Effect of Polyphenols on Viscosity of Emulsions

The effect of 0.5% GTE, GSE, CAT and 1% CAT on emulsion viscosity is seen in Figure 4.19 for CPEC, FPECn and FPECc. The results showed that the addition of polyphenols caused a significant reduction in the viscosity of all CPEC emulsions. The addition of GTE and GSE at 0.5% caused 24 and 13% reduction in CPEC emulsion viscosity. The effect of addition of GTE and GSE was different for fig pectins. For FPECn emulsions slight changes were observed with GSE causing 19% reduction GTE causing 11% increase in the viscosity. On the other hand, the addition of both GTE and GSE increased the viscosity of the FPECc emulsions while only the change caused by GSE was significant with 42% increase.

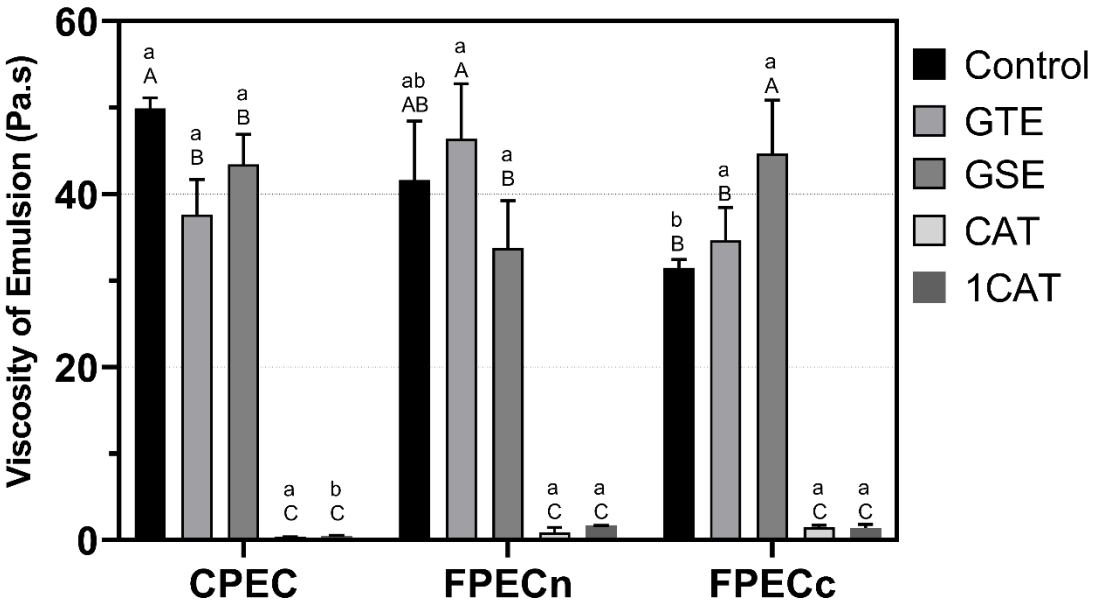

Figure 4.19. Viscosities of pectin (0.5%)-polyphenol (0.5-1%) emulsions stored for 1 day at room temperature (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples of pectin. Lower case letters are for values of the same pectin with various phenolic concentrations).

The pectin extracts are complex molecules with molecular interactions that are not yet revealed completely hence the difference among fig pectins viscosities to GTE and GSE could be due to an unknown molecular mechanism. On the other hand, addition of CAT at 0.5 and 1% lowered the viscosity of all pectins significantly when compared to control emulsions.

The control emulsion viscosity of FPECc was lower than both CPEC and FPECn emulsion viscosity while the RG-I ratio for FPECn was significantly higher than CPEC and FPECc pectins. It should be noted that the hydrocolloids like pectin increase the viscosity of solutions due to the entanglements formed among their linear HG chains (Sousa et al. 2015). It appears that the GTE and GSE showed limited changes in the linearity of HGs that are not involved in emulsion (those suspended in aqueous phase). In contrast, the CAT seemed to interact effectively with all HG chains (both suspended free ones and those coalesced around lipid droplets) and spoiled their linear arrangements that are essential to increase viscosity of emulsion. (Z. Liu, Guo, and Meng 2020) did not find a significant effect of ferulic acid on viscosities of oil-in-water emulsions stabilized by citrus, apple and sugar beet pectins. However, these researchers used a triglyceride (C8/C10 fatty acid ratio 60:40) to form their emulsions and studied with a ferulic acid concentration at maximum 0.3%. Thus, it is not very meaningful to compare their results with those of the current study.

4.8. Polyphenol Content of Emulsions During Storage

The polyphenol content of various CPEC emulsions during storage are given in Figure 4.20. As expected, the control CPEC emulsions contained insignificant amounts of polyphenols. For emulsions with 0.5% GSE, GTE or CAT the highest initial polyphenol content was determined for CAT added emulsion followed descending order by GSE and GTE added emulsions at day 1. This result was expected since total polyphenol contents of CAT and GSE preparations used were almost 2.4 and 2.1-fold higher than that of GTE preparation, respectively. Emulsions prepared with 0.5% GSE and CAT showed significant reduction in polyphenol content up to 5th day of storage but maintained their remaining polyphenol content between days 5 and 14. In contrast, the GTE in emulsions had a high stability and showed a limited reduction in its polyphenol content. The CPEC

emulsions prepared with 0.5% GTE, GSE and CAT showed almost 18, 42 and 48% overall reduction in their initial polyphenol content at the end of 14-days storage.

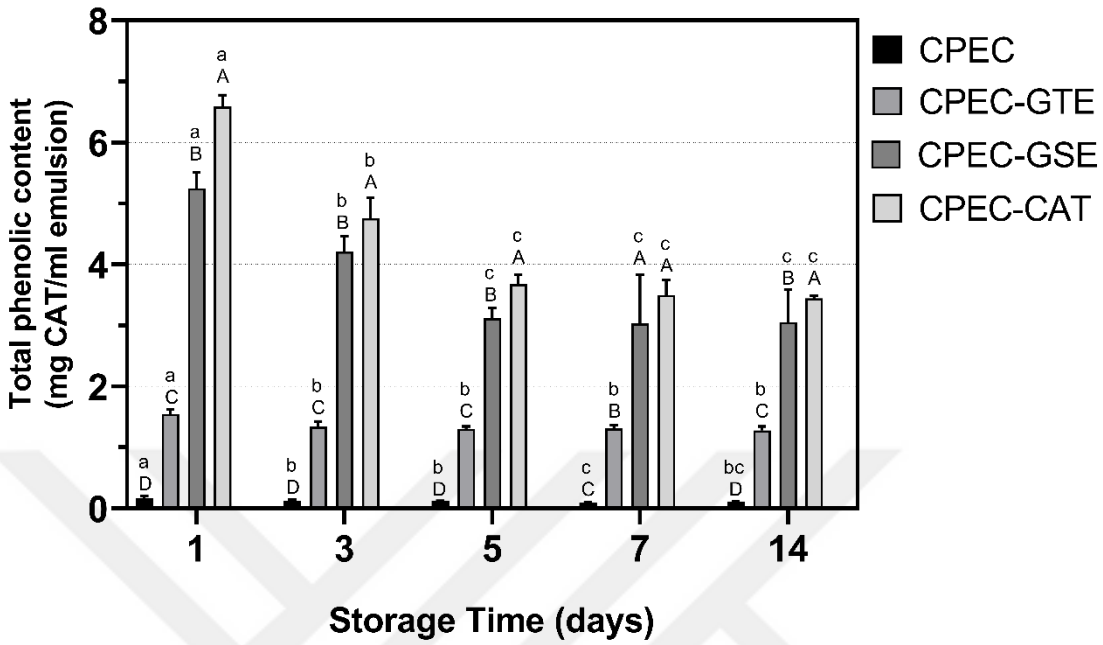

Figure 4.20. The change in phenolic content of 0.5% CPEC- 0.5% polyphenol emulsions during 14 days of storage (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

The change in the phenolic content of FPECn and FPECc emulsions during storage are given in Figure 4.21 and 4.22. Similar to CPEC emulsions, a significantly higher phenolic content was determined for GSE than GTE added FPECn and FPECc emulsions. The addition of 0.5% GTE and GSE caused 9.6 and 32-fold increase in FPECn, and 9.5 and 23.4-fold increase in FPECc emulsions at day 1, respectively. However, the phenolic contents for 0.5% GTE and GSE added emulsions reduced 13 and 30% for FPECn, and 26 and 11% for FPECc at the end of 14 days of storage, respectively. Interestingly, the GSE polyphenols showed higher reduction in FPECn than in FPECc. In contrast, the GTE showed higher reduction in FPECc than in FPECn. These differences in amount of polyphenol reduction should be originated from compositional differences in FPECn and FPECc that affect the amounts of bind and soluble polyphenols in the emulsion. Moreover, the reduction of polyphenol content by storage might also be related to degradation of polyphenols due to their low stability in the emulsions.

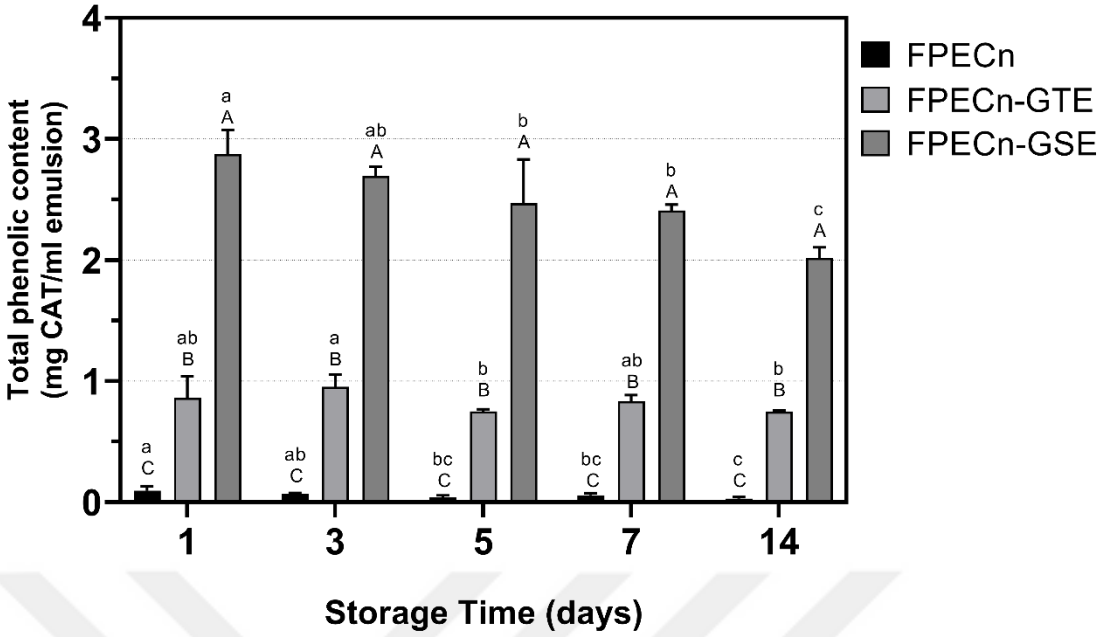

Figure 4.21. The change in phenolic content of 0.5% FPECn- 0.5% polyphenol emulsions during 14 days of storage (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

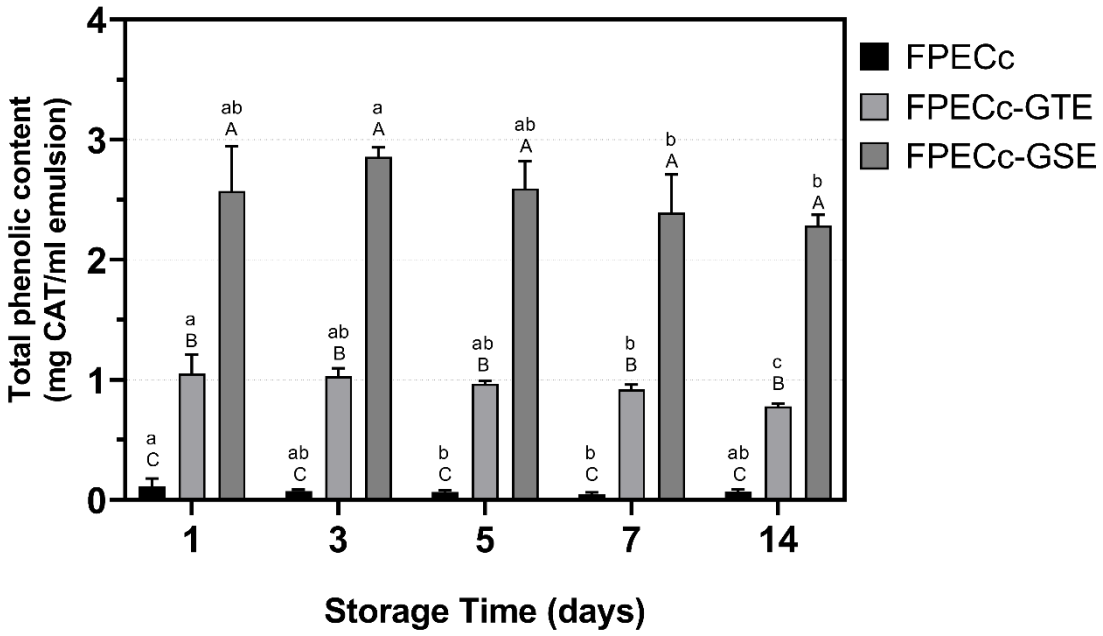

Figure 4.22. The change in phenolic content of 0.5% FPECc- 0.5% polyphenol emulsions during 14 days of storage (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

The comparison of phenolic contents of different emulsions clearly showed that the CPEC emulsions contained significantly higher soluble polyphenols than FPECn and FPECc emulsions. Thus, it appeared that FPECn and FPECc bind the added polyphenols and prevented their measurement during polyphenol determination. The protein content of FPECn and FPECc preparations were also considerably higher than that of commercial CPEC. Thus, it is possible that the polyphenols added into fig pectin emulsions were bind also by the proteins. The protein-polyphenol binding occurs mainly by hydrogen bonds formed between hydroxyl groups of phenolic compounds and carbonyl groups of protein (Arcan and Yemenicioğlu 2011). The hydrophobic interactions formed between aromatic rings (e.g., A and C rings of flavonoids) of polyphenols and hydrophobic methyl groups of pectin are accepted as the primary mechanism of polyphenol-pectin complexation (Liu et al., 2020; Tang et al., 2020). Then, extensive hydrogen bonds forming between polyphenols and pectin cause stabilization of the complexes (X. Liu, Le Bourvellec, and Renard 2020). Z. Liu et al., (2020) showed that the interfacial concentration of citrus pectin in the oil-in-water emulsion increased 2.7-fold by addition of ferulic acid. These authors hypothesized that the presence of ferulic acid caused pectin multilayer formation around lipid droplets by bridging pectin molecules. This hypothesis might be used to explain greater reductions of electro negative charges in GSE and CAT stabilized emulsions than the other emulsions during storage. It is likely that the GSE and GTE affected emulsion stability mainly by inducing formation of multilayer pectin molecules around lipid droplets. However, it was also hypothesized that the phenolic compounds absorbed at the interface might have also interacted with pectin molecules to increase the density (packing) of absorbed pectin layer at the oil-water interface (Z. Liu, Guo, and Meng 2020).

4.9. Antioxidant Activity of Emulsions During Storage

The changes that occur in the antioxidant activity of emulsions during storage period are presented in Figure 4.23, 4.24 and 4.25 for CPEC, FPECn and FPECc, respectively. The results of antioxidant activity measurements were parallel with the initial total phenolic contents of emulsions. The control emulsions showed very limited antioxidative activity that possibly originates from the residual amphiphilic polyphenols of olive oil.

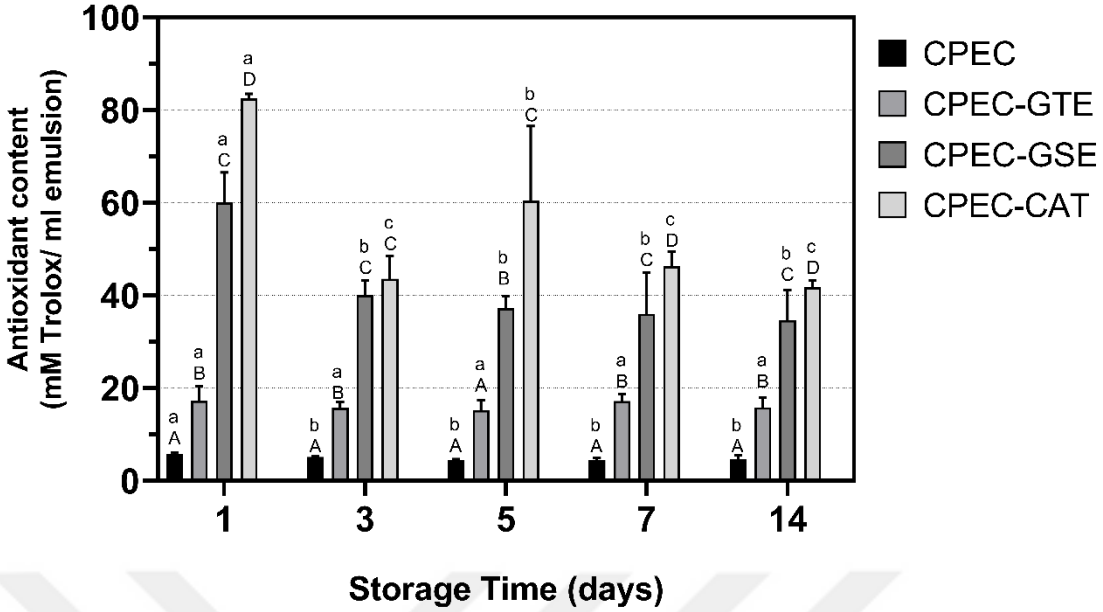

Figure 4.23. The change in antioxidant activity of 0.5% CPEC- 0.5% polyphenol emulsions during 14 days of storage (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

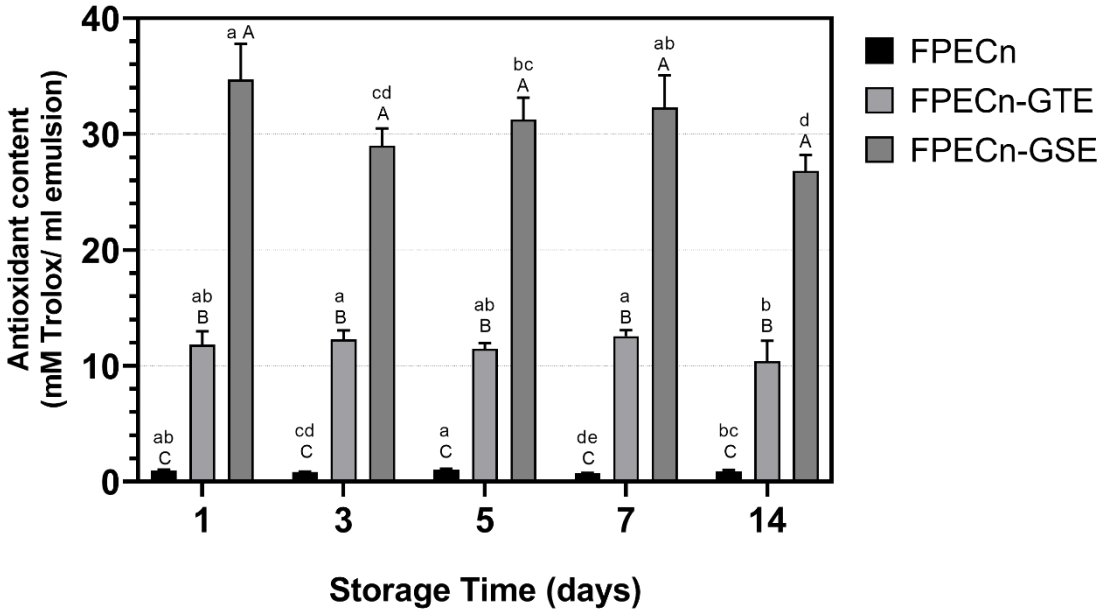

Figure 4.24. The change in antioxidant activity of 0.5% FPECn- 0.5% polyphenol emulsions during 14 days of storage (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

The pectin itself also shows some limited free radical scavenging activity originating from its polysaccharide structure (e.g., -OH and -COOH groups) (Gharibzahedi, Smith, and Guo 2019; W. Wang et al. 2016) and bound antioxidant components such as Maillard reaction products, proteins and polyphenols (Domínguez Avila et al. 2018).

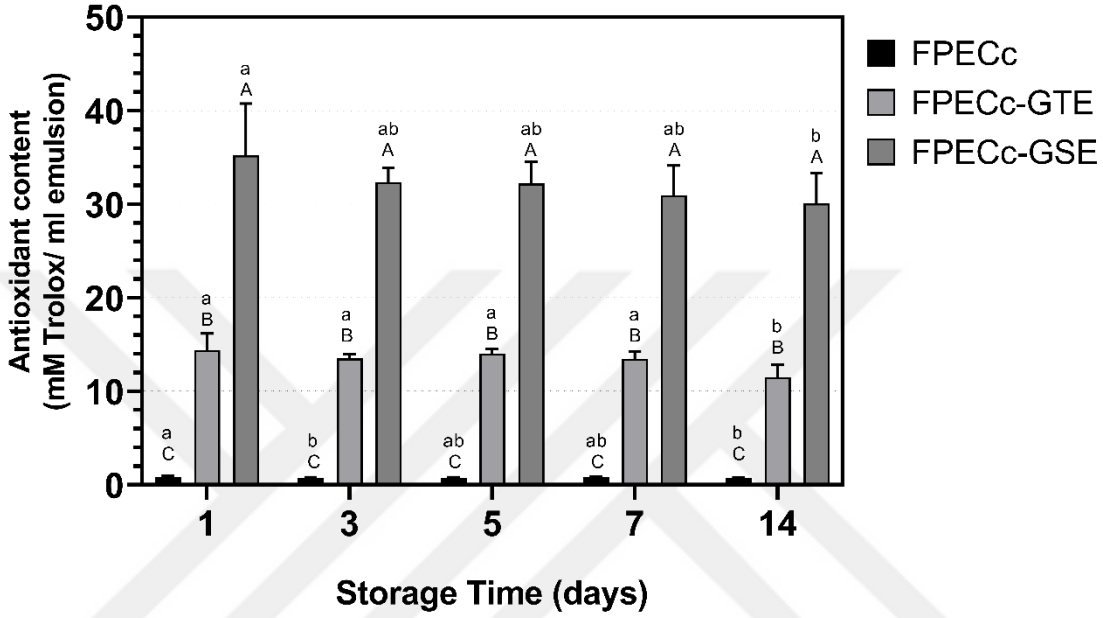

Figure 4.25. The change in antioxidant activity of 0.5% FPECc- 0.5% polyphenol emulsions during 14 days of storage (Different letters indicate significantly different values at $P < 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points).

The results obtained for FPECn and FPECc emulsions were almost identical during the storage time. A significant decrease in the antioxidant activity was observed for both fig pectin emulsions with the addition of 0.5% GSE. The reductions in antioxidant activities of FPECn and FPECc emulsion with GSE were 23 and 14% within 14 days of storage, respectively. Similar to polyphenol contents of emulsions, antioxidant activity measured in CPEC emulsions was significantly higher than those in fig pectin emulsions. This result was expected since the antioxidant activity measured is directly related with the polyphenol content of emulsions.

CHAPTER 5

CONCLUSIONS

This thesis investigated the olive oil-in-water emulsion stabilizing capacities of citrus pectin and novel pectins extracted from whole sun-dried Sarılop and Siyah Orak fig cultivars. For the first time in literature, contributions of different phenolic extracts and a basic flavonoid catechin have been investigated with detailed storage and characterization studies. The major conclusions reached by laboratory tests are as follows; (1) the pectins extracted from sun-dried Sarılop and Siyah Orak fig varieties showed similar emulsion stabilizing capacity with commercial citrus pectin, (2) emulsion stabilizing capacity of fig pectins was improved only by catechin while grapeseed and green tea extracts showed almost no positive contribution on emulsion stability of fig pectins, (3) addition of both grapeseed phenolic extract and catechin improved the emulsion stabilizing capacity of citrus pectin while green tea extract caused limited contribution in emulsion stability of citrus pectin, (4) particle size analysis suggested that the improved emulsion stabilizing activity with grapeseed extract and catechin might be due to reduced droplet size of emulsions in presence of these polyphenols, (5) the addition of polyphenols improved the antioxidant capacity of all emulsions, but emulsions with grapeseed extract and catechin caused significantly higher antioxidant capacity than those with green tea extract, (6) emulsions prepared with commercial citrus pectin showed significantly higher soluble phenolic content and antioxidant activity than those of fig pectins that apparently bind and immobilized polyphenols. This work clearly showed that GSE and CAT stabilized CPEC emulsions, and CAT stabilized FPECn or FPECc emulsions have a great potential to develop novel antioxidant olive oil-based functional foods. Further studies are needed to conduct extensive product development studies with the characterized olive-oil-in-water emulsions, but it appears that the polyphenol added pectin stabilized emulsions could easily be transformed into series of novel creamy functional olive oil products, salad dressings, and meal sauces by addition of different seasonings, flavouring substances and ingredients. However, these studies should be backed not only by extensive stability tests in food, but also proper bioavailability and bioaccessibility tests with suitable cell and animal models.

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APPENDICES

APPENDIX A. The Standard Curves Used for The Spectrophotometric Methods

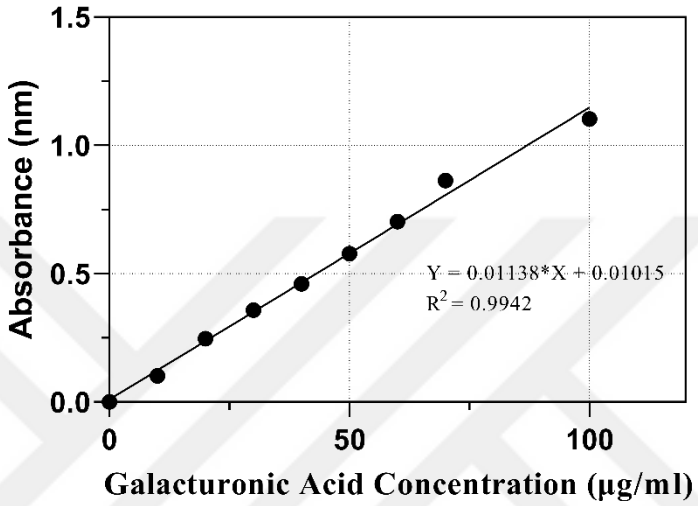

Figure A1. The standard curve prepared with galacturonic acid for galacturonic acid content measurement

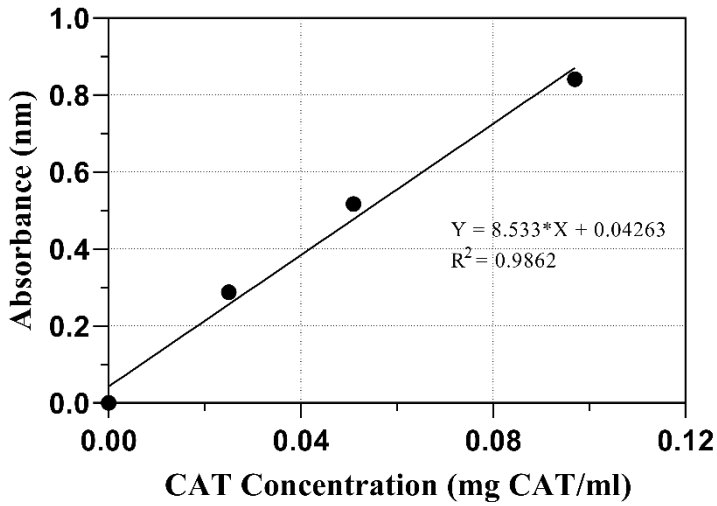

Figure A2. The standard curve prepared with catechin for total phenolic content measurement

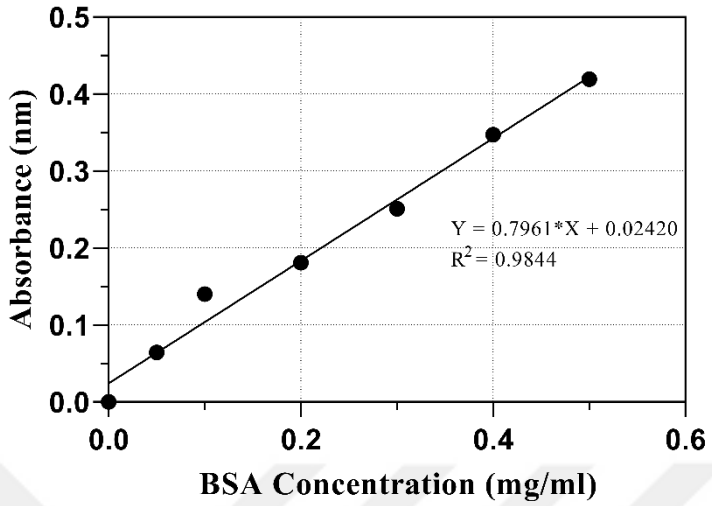

Figure A3. The standard curve prepared with bovine serum albumin for total protein content measurement

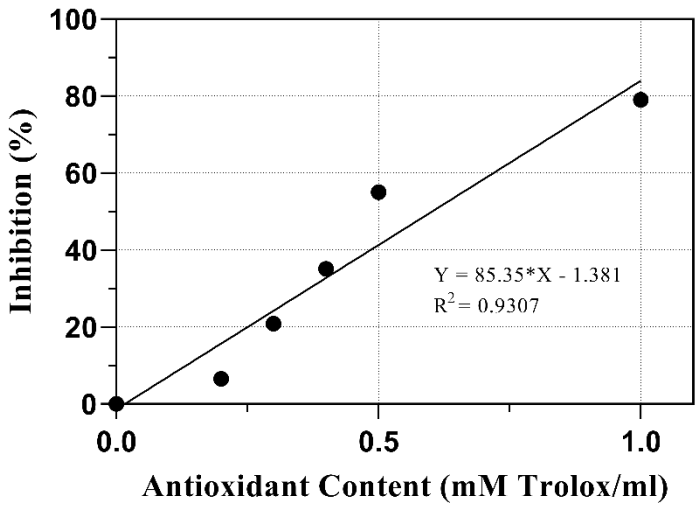

Figure A4. The standard curve prepared with Trolox for antioxidant activity determination

APPENDIX B. The Data Tables of The Experimental Findings

Table B1. The data table for emulsion stability tests conducted for CPEC

Time(day) /CPEC concentration (%, w/v)	**0.125%**	**0.25%**	**0.5%**	**1%**
0	$92.30\pm1.70^{a,B}$	$100\pm0^{a,A}$	$100\pm0^{a,A}$	$100\pm0^{a,A}$
0.25	$73.05\pm0.35^{b,C}$	$83.3\pm4.38^{b,B}$	$100\pm0^{a,A}$	$100\pm0^{a,A}$
1	$58.25\pm2.47^{c,B}$	$63.5\pm1.48^{c,B}$	$64.20\pm2.40^{b,B}$	$95.95\pm5.73^{a,A}$
3	$58.25\pm2.47^{c,B}$	$63.0\pm0.71^{c,B}$	$64.20\pm2.40^{b,B}$	$90.60\pm8.06^{ab,A}$
5	$58.25\pm2.47^{c,B}$	$63.0\pm0.71^{c,B}$	$64.20\pm2.40^{b,B}$	$88.75\pm7.14^{ab,A}$
7	$56.05\pm0.64^{c,C}$	$63.0\pm0.71^{c,B}$	$64.20\pm2.40^{b,B}$	$81.85\pm2.62^{b,A}$
14	$53.90\pm2.40^{c,C}$	$59.2\pm1.34^{c,CB}$	$64.20\pm2.40^{b,B}$	$81.30\pm1.84^{b,A}$

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B2. The data table for emulsion stability tests conducted for FPECn

Time(day) /FPECn concentration (%, w/v)	**0.125%**	**0.25%**	**0.5%**	**1%**
0	$66.90\pm1.41^{a,C}$	$73.20\pm2.55^{a,B}$	$74.35\pm0.21^{a,B}$	$100\pm0^{a,A}$
0.25	$74.10\pm1.27^{b,B}$	$72.70\pm1.84^{a,B}$	$77.00\pm0.57^{b,B}$	$92.95\pm3.18^{b,A}$
1	$51.80\pm0.85^{c,C}$	$66.95\pm7.14^{a,B}$	$71.10\pm1.27^{c,B}$	$82.35\pm1.34^{c,A}$
3	$51.80\pm0.85^{c,B}$	$62.20\pm13.86^{a,AB}$	$71.10\pm1.27^{c,AB}$	$78.80\pm3.68^{cd,A}$
5	$51.80\pm0.85^{c,B}$	$62.20\pm13.86^{a,AB}$	$71.10\pm1.27^{c,AB}$	$75.85\pm1.20^{d,A}$
7	$51.80\pm0.85^{c,C}$	$59.20\pm9.62^{a,BC}$	$67.90\pm0.28^{d,AB}$	$75.85\pm1.20^{d,A}$
14	$51.80\pm0.85^{c,C}$	$59.20\pm9.62^{a,BC}$	$66.85\pm1.77^{d,AB}$	$75.85\pm1.20^{d,A}$

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B3. The data table for emulsion stability tests conducted for FPECc

Time(day) /FPECc concentration (%, w/v)	**0.125%**	**0.25%**	**0.5%**	**1%**
0	84.90±0.71^{a,C}	98.55±0.49^{a,B}	97.75±0.07^{a,B}	100±0^{a,A}
0.25	68.80±0.00^{b,C}	80.10±3.96^{b,B}	85.80±1.98^{b,B}	97.15±0.78^{a,A}
1	60.40±2.97^{c,BC}	57.75±4.60^{c,C}	68.75±0.21^{c,B}	86.15±3.46^{b,A}
3	58.30±0.00^{c,C}	59.90±3.68^{c,C}	67.65±1.34^{c,B}	82.75±1.34^{bc,A}
5	58.30±0.00^{c,C}	59.90±3.68^{c,C}	67.05±0.49^{c,B}	82.75±1.34^{bc,A}
7	58.30±0.00^{c,C}	58.75±2.05^{c,C}	67.05±0.49^{c,B}	81.60±2.97^{bc,A}
14	58.30±0.00^{c,C}	58.75±2.05^{c,C}	67.05±0.49^{c,B}	81.05±2.19^{c,A}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B4. The data table for emulsion stability tests conducted for CPEC emulsions with the addition of GTE

Time(day) /GTE concentration (%, w/v)	**Control**	**0.05%**	**0.25%**	**0.50%**
0	100.00±0^{a,A}	100.00±0^{a,A}	100.00±0^{a,A}	100.00±0^{a,A}
0.25	100.00±0^{a,A}	98.50±1.91^{a,A}	99.50±0.58^{a,A}	90.50±0.58^{a,A}
1	64.20±2.40^{b,B}	89.50±10.79^{b,A}	90.13±10.57^{ab,A}	83.63±8.26^{b,A}
3	64.20±2.40^{b,B}	79.13±6.84^{c,A}	80.75±10.24^{bc,A}	73.50±4.73^{c,AB}
5	64.20±2.40^{b,B}	77.13±5.51^{cd,A}	77.75±7.50^{c,A}	69.75±2.87^{cd,AB}
7	64.20±2.40^{b,B}	76.88±5.27^{cd,A}	76.25±5.68^{c,A}	68.50±2.52^{cd,B}
14	64.20±2.40^{b,B}	70.00±3.65^{d,AB}	73.75±3.30^{c,A}	65.50±5.51^{d,B}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B5. The data table for emulsion stability tests conducted for FPECn emulsions with the addition of GTE

Time(day) /GTE concentration (%, w/v)	**Control**	**0.05%**	**0.25%**	**0.50%**
0	74.35±0.21^{a,B}	99.25±0.96^{a,A}	99.00±1.41^{a,A}	98.88±1.03^{a,A}
0.25	77.00±0.57^{b,B}	89.00±7.53^{b,A}	75.25±4.03^{b,B}	81.00±1.15^{b,B}
1	71.10±1.27^{c,B}	83.63±3.77^{b,A}	69.00±4.76^{b,B}	74.00±4.62^{c,B}
3	71.10±1.27^{c,B}	83.13±3.71^{b,A}	68.75±4.99^{b,B}	72.88±4.25^{c,B}
5	71.10±1.27^{c,B}	83.00±3.83^{b,A}	68.50±5.26^{b,B}	72.88±4.25^{c,B}
7	67.90±0.28^{d,B}	83.00±3.83^{b,A}	68.50±5.26^{b,B}	72.88±4.25^{c,B}
14	66.85±1.77^{d,A}	74.50±7.55^{c,A}	68.50±5.26^{b,A}	67.13±2.66^{d,A}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B6. The data table for emulsion stability tests conducted for FPECc emulsions with the addition of GTE

Time(day) /GTE concentration (%, w/v)	**Control**	**0.05%**	**0.25%**	**0.50%**
0	97.75±0.07^{a,A}	99.88±0.25^{a,A}	99.25±1.50^{a,A}	97.75±2.63^{a,A}
0.25	85.80±1.98^{b,A}	85.25±0.96^{b,A}	83.38±2.50^{b,A}	77.25±4.57^{b,B}
1	68.75±0.21^{c,B}	76.75±2.22^{c,A}	73.50±4.43^{c,AB}	69.63±4.23^{c,B}
3	67.65±1.34^{c,B}	74.50±3.42^{c,A}	69.00±2.00^{d,AB}	69.75±5.06^{c,AB}
5	67.05±0.49^{c,B}	74.50±3.42^{c,A}	69.00±2.00^{d,AB}	69.25±4.99^{c,AB}
7	67.05±0.49^{c,B}	74.50±3.42^{c,A}	68.75±2.22^{d,B}	69.50±4.80^{c,AB}
14	67.05±0.49^{c,B}	74.25±3.30^{c,A}	69.50±1.91^{d,AB}	69.75±5.06^{c,AB}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B7. The data table for emulsion stability tests conducted for CPEC emulsions with the addition of GSE

Time(day) /GSE concentration (%, w/v)	**Control**	**0.05%**	**0.25%**	**0.50%**
0	$100.00\pm0^{a,A}$	$100.00\pm0^{a,A}$	$100.00\pm0^{a,A}$	$100.00\pm0^{a,A}$
0.25	$100.00\pm0^{a,A}$	$100.00\pm0^{a,A}$	$100.00\pm0^{a,A}$	$100.00\pm0^{a,A}$
1	$64.20\pm2.40^{b,B}$	$90.13\pm11.49^{ab,A}$	$100.00\pm0^{a,A}$	$100.00\pm0^{a,A}$
3	$64.20\pm2.40^{b,C}$	$86.00\pm10.98^{bc,B}$	$97.50\pm3.00^{ab,A}$	$99.00\pm1.41^{a,A}$
5	$64.20\pm2.40^{b,C}$	$83.25\pm10.24^{bc,B}$	$96.50\pm4.12^{ab,A}$	$98.00\pm2.45^{ab,A}$
7	$64.20\pm2.40^{b,C}$	$80.63\pm9.72^{bc,B}$	$95.25\pm5.50^{ab,A}$	$97.63\pm3.09^{ab,A}$
14	$64.20\pm2.40^{b,B}$	$74.25\pm6.45^{c,B}$	$92.00\pm9.38^{b,A}$	$94.25\pm6.95^{b,A}$

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B8. The data table for emulsion stability tests conducted for FPECn emulsions with the addition of GSE

Time(day) /GSE concentration (%, w/v)	**Control**	**0.05%**	**0.25%**	**0.50%**
0	$74.35\pm0.21^{a,C}$	$90.50\pm4.73^{a,B}$	$97.88\pm2.46^{a,A}$	$100.00\pm0.00^{a,A}$
0.25	$77.00\pm0.57^{b,A}$	$78.50\pm5.80^{b,A}$	$81.00\pm8.29^{b,A}$	$84.25\pm11.27^{b,A}$
1	$71.10\pm1.27^{c,A}$	$73.38\pm6.60^{b,A}$	$72.75\pm7.80^{b,A}$	$73.38\pm8.52^{bc,A}$
3	$71.10\pm1.27^{c,A}$	$72.63\pm5.94^{b,A}$	$72.13\pm7.66^{b,A}$	$71.50\pm7.55^{c,A}$
5	$71.10\pm1.27^{c,A}$	$73.38\pm6.60^{b,A}$	$72.00\pm7.53^{b,A}$	$71.50\pm7.55^{c,A}$
7	$67.90\pm0.28^{d,A}$	$73.63\pm6.85^{b,A}$	$71.25\pm7.27^{b,A}$	$71.63\pm7.70^{c,A}$
14	$66.85\pm1.77^{d,A}$	$73.50\pm6.61^{b,A}$	$71.25\pm7.27^{b,A}$	$71.75\pm7.85^{c,A}$

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B9. The data table for emulsion stability tests conducted for FPECc emulsions with the addition of GTE

Time(day) /GSE conc	**Control**	**0.05%**	**0.25%**	**0.50%**
0	97.75±0.07^{a,A}	98.38±1.89^{a,A}	98.00±1.15^{a,A}	99.50±0.58^{a,A}
0.25	85.80±1.98^{b,A}	85.25±5.78^{b,A}	85.50±5.26^{b,A}	83.25±6.85^{b,A}
1	68.75±0.21^{c,C}	78.63±0.95^{c,A}	73.00±4.76^{c,BC}	76.50±3.00^{c,AB}
3	67.65±1.34^{c,B}	74.25±3.10^{c,A}	71.25±4.43^{c,AB}	76.50±3.00^{c,A}
5	67.05±0.49^{c,B}	74.50±3.42^{c,A}	71.50±4.12^{c,AB}	75.50±2.65^{c,A}
7	67.05±0.49^{c,B}	74.25±3.10^{c,A}	71.25±4.43^{c,AB}	76.00±2.94^{c,A}
14	67.05±0.49^{c,B}	74.25±3.10^{c,A}	71.50±4.12^{c,AB}	75.75±2.36^{c,A}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B10. The data table for emulsion stability tests conducted for CPEC emulsions with the addition of CAT

Time(day) /CAT concentration (%, w/v)	**Control**	**0.50%**	**1.00%**
0	100.00±0^{a,A}	100.00±0^{a,A}	100.00±0^{a,A}
0.25	100.00±0^{a,A}	100.00±0^{a,A}	100.00±0^{a,A}
1	64.20±2.40^{b,B}	98.00±0.00^{b,A}	100.00±0^{a,A}
3	64.20±2.40^{b,C}	89.17±0.76^{c,B}	100.00±0^{a,A}
5	64.20±2.40^{b,C}	85.00±1.00^{d,B}	100.00±0^{a,A}
7	64.20±2.40^{b,C}	77.00±1.00^{e,B}	100.00±0^{a,A}
14	64.20±2.40^{b,C}	72.67±1.15^{f,B}	94.67±1.15^{b,A}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B11. The data table for emulsion stability tests conducted for FPECn emulsions with the addition of CAT

Time(day) /CAT concentration (%, w/v)	**Control**	**0.50%**	**1.00%**
0	74.35±0.21^{a,B}	100.00±0^{a,A}	100.00±0^{a,A}
0.25	77.00±0.57^{b,C}	89.33±1.15^{b,B}	100.00±0^{a,A}
1	71.10±1.27^{c,C}	80.83±0.76^{c,B}	90.33±2.52^{b,A}
3	71.10±1.27^{c,C}	80.83±0.76^{c,B}	88.83±1.44^{b,A}
5	71.10±1.27^{c,C}	80.83±0.76^{c,B}	88.83±1.44^{b,A}
7	67.90±0.28^{d,C}	80.50±1.32^{c,B}	88.67±1.15^{b,A}
14	66.85±1.77^{d,C}	80.33±1.53^{c,B}	88.67±1.15^{b,A}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B12. The data table for emulsion stability tests conducted for FPECc emulsions with the addition of CAT

Time(day) /CAT concentration (%, w/v)	**Control**	**0.50%**	**1.00%**
0	97.75±0.07^{a,B}	100.00±0^{a,A}	100.00±0^{a,A}
0.25	85.80±1.98^{b,C}	90.17±0.29^{b,B}	99.00±0.50^{b,A}
1	68.75±0.21^{c,C}	79.50±0.50^{c,B}	89.33±1.15^{c,A}
3	67.65±1.34^{c,C}	78.50±0.50^{cd,B}	86.00±0.00^{d,A}
5	67.05±0.49^{c,C}	77.00±1.32^{cd,B}	86.00±0.00^{d,A}
7	67.05±0.49^{c,C}	76.17±1.89^{de,B}	85.33±0.58^{d,A}
14	67.05±0.49^{c,C}	73.50±3.28^{e,B}	84.33±0.58^{e,A}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B13. The data table for particle size analyses results conducted for CPEC emulsions with various phenolic compounds

Time (day)/ sample	0.5% CPEC	0.5% CPEC-0.5% GTE	0.5% CPEC-0.5% GSE	0.5% CPEC-0.5% CAT	0.5% CPEC-1% CAT
1	96.03±9.62^{a,B}	65.71±5.48^{a,A}	22.44±3.53^{a,D}	37.74±3.89^{a,C}	31.34±1.62^{a,CD}
7	78.82±16.86^{a,A}	65.12±2.36^{a,B}	26.50±3.15^{a,D}	44.56±3.07^{b,C}	33.50±2.02^{a,D}
14	78.38±15.75^{a,A}	63.04±1.16^{a,A}	17.66±0.85^{b,C}	22.90±2.69^{c,BC}	34.32±2.02^{a,B}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B14. The data table for particle size analyses results conducted for FPECn emulsions with various phenolic compounds

Time(day)/ sample	0.5% FPECn	0.5% FPECn-0.5% GTE	0.5% FPECn-0.5% GSE	0.5% FPECn-0.5% CAT	0.5% FPECn-1% CAT
1	30.21±5.77^{a,A}	24.39±3.92^{a,B}	13.03±3.70^{a,C}	9.18±1.08^{a,CD}	3.84±0.18^{a,D}
7	26.45±2.85^{ab,A}	10.72±4.51^{b,B}	10.41±1.63^{a,B}	8.67±0.75^{a,BC}	4.33±0.38^{a,C}
14	21.20±6.81^{b,A}	18.98±2.56^{c,A}	10.34±1.40^{a,B}	8.85±1.23^{a,BC}	4.09±0.55^{a,C}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B15. The data table for particle size analyses results conducted for FPECc emulsions with various phenolic compounds

Time(day)/sample	0.5% FPECc	0.5% FPECc-0.5% GTE	0.5% FPECc-0.5% GSE	0.5% FPECc-0.5% CAT	0.5% FPECc-1% CAT
1	30.32±7.21^{a,A}	20.08±4.72^{a,B}	14.23±3.34^{a,C}	13.37±2.65^{a,C}	4.16±0.88^{a,D}
7	15.65±2.78^{b,AB}	16.02±6.25^{a,A}	11.91±1.35^{a,AB}	11.30±1.49^{a,B}	4.23±0.43^{a,C}
14	15.43±2.11^{b,A}	12.70±6.33^{a,AB}	12.00±1.29^{a,AB}	10.20±0.61^{a,AB}	10.08±0.51^{b,B}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B16. The data table for zeta potential analyses results conducted for CPEC emulsions with various phenolic compounds

Time(day)/ sample	0.5% CPEC	0.5% CPEC-0.5% GTE	0.5% CPEC-0.5% GSE	0.5% CPEC-0.5% CAT	0.5% CPEC-1% CAT
1	-26.91±1.64^{c,A}	-26.79±0.75^{b,A}	-29.17±0.45^{c,B}	-29.48±0.63^{c,B}	-27.50±0.55^{c,A}
7	-21.52±0.48^{a,B}	-22.47±1.08^{a,C}	-21.83±0.27^{b,BC}	-20.21±0.61^{b,A}	-21.64±0.47^{b,B}
14	-23.50±1.61^{b,D}	-21.63±1.21^{a,C}	-18.47±0.50^{a,B}	-17.10±0.28^{a,A}	-19.53±0.48^{a,B}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B17. The data table for zeta potential analyses results conducted for FPECn emulsions with various phenolic compounds

Time(day) /sample	0.5% FPECn	0.5% FPECn- 0.5% GTE	0.5% FPECn- 0.5% GSE
1	-27.11±1.48^{a,A}	-27.42±0.71^{a,A}	-27.72±2.65^{a,A}
7	-25.55±0.52^{a,A}	-27.27±1.14^{a,A}	-31.04±1.74^{a,B}
14	-25.98±0.43^{a,A}	-25.70±0.74^{a,A}	-27.12±1.55^{a,A}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B18. The data table for zeta potential analyses results conducted for FPECc emulsions with various phenolic compounds

Time(day) /sample	0.5% FPECc	0.5% FPECc- 0.5% GTE	0.5% FPECc- 0.5% GSE
1	-25.91±0.35^{ab,A}	-29.32±0.33^{b,B}	-26.21±0.58^{b,A}
7	-26.38±0.77^{b,B}	-26.10±0.79^{a,B}	-20.35±1.16^{a,A}
14	-25.23±0.12^{a,A}	-26.53±0.70^{a,B}	-26.16±0.43^{b,AB}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B19. The data table for antioxidant activity results of CPEC-phenolic compound emulsions

Time (day) /sample	0.5% CPEC	0.5% CPEC-0.5% GTE	0.5% CPEC-0.5% GSE	0.5% CPEC-0.5% CAT
1	5.80±0.31^{a,D}	17.30±3.01^{a,C}	60.02±13.80^{a,B}	82.48±1.01^{a,A}
3	5.10±0.14^{b,C}	15.70±1.22^{a,B}	40.03±3.71^{b,A}	43.58±4.95^{c,A}
5	4.43±0.23^{b,C}	15.22±2.14^{a,C}	37.24±8.11^{b,B}	60.43±16.10^{b,A}
7	4.43±0.49^{b,D}	17.17±1.56^{a,C}	36.02±8.42^{b,B}	46.34±3.06^{c,A}
14	4.67±0.79^{b,D}	15.79±2.15^{a,C}	34.65±6.66^{b,B}	41.74±1.50^{c,A}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B20. The data table for antioxidant activity results of FPECn-phenolic compound emulsions

Time (day) /sample	0.5% FPECn	0.5% FPECn- 0.5% GTE	0.5% FPECn- 0.5% GSE
1	0.97±0.07^{ab,C}	11.81±1.17^{ab,B}	34.72±3.44^{a,A}
3	0.83±0.04^{cd,C}	12.27±0.78^{a,B}	29.01±1.35^{cd,A}
5	1.02±0.11^{a,C}	11.46±0.48^{ab,B}	31.25±1.01^{bc,A}
7	0.72±0.03^{de,C}	12.55±0.53^{a,B}	32.31±2.48^{ab,A}
14	0.88±0.14^{bc,C}	10.39±1.75^{b,B}	26.81±1.62^{d,A}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B21. The data table for antioxidant activity results of FPECn-phenolic compound emulsions

Time (day) /sample	0.5% FPECc	0.5% FPECc- 0.5% GTE	0.5% FPECc- 0.5% GSE
1	0.86±0.13^{a,C}	14.42±1.75^{a,B}	35.24±4.44^{a,A}
3	0.69±0.12^{b,C}	13.53±0.42^{a,B}	32.36±1.29^{ab,A}
5	0.75±0.07^{ab,C}	14.05±0.50^{a,B}	32.20±1.91^{ab,A}
7	0.80±0.04^{ab,C}	13.48±0.75^{a,B}	30.97±3.35^{ab,A}
14	0.71±0.02^{b,C}	11.53±1.28^{b,B}	30.10±3.22^{b,A}

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B22. The data table for total phenolic content determination results of CPEC-phenolic compound emulsions

Time (day) /sample	0.5% CPEC	0.5% CPEC-0.5% GTE	0.5% CPEC-0.5% GSE	0.5% CPEC-0.5% CAT
1	$0.17\pm0.04^{a,D}$	$1.55\pm0.08^{a,C}$	$5.25\pm0.27^{a,B}$	$6.59\pm0.19^{a,A}$
3	$0.12\pm0.03^{b,D}$	$1.34\pm0.08^{b,C}$	$4.21\pm0.25^{b,B}$	$4.76\pm0.34^{b,A}$
5	$0.12\pm0.00^{b,D}$	$1.31\pm0.04^{b,C}$	$3.12\pm0.17^{c,B}$	$3.67\pm0.16^{c,A}$
7	$0.09\pm0.01^{c,C}$	$1.31\pm0.05^{b,B}$	$3.03\pm0.80^{c,A}$	$3.50\pm0.25^{c,A}$
14	$0.10\pm0.01^{bc,D}$	$1.28\pm0.06^{b,C}$	$3.05\pm0.53^{c,B}$	$3.44\pm0.04^{c,A}$

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B23. The data table for total phenolic content determination results of FPECn-phenolic compound emulsions

Time (day) /sample	0.5% FPECn	0.5% FPECn- 0.5% GTE	0.5% FPECn-0.5% GSE
1	$0.09\pm0.04^{a,C}$	$0.86\pm0.18^{ab,B}$	$2.88\pm0.20^{a,A}$
3	$0.07\pm0.01^{ab,C}$	$0.95\pm0.10^{a,B}$	$2.70\pm0.08^{ab,A}$
5	$0.04\pm0.01^{bc,C}$	$0.75\pm0.02^{b,B}$	$2.47\pm0.36^{b,A}$
7	$0.06\pm0.02^{bc,C}$	$0.83\pm0.05^{ab,B}$	$2.41\pm0.05^{b,A}$
14	$0.03\pm0.01^{c,C}$	$0.75\pm0.01^{b,B}$	$2.02\pm0.09^{c,A}$

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B24. The data table for total phenolic content determination results of FPECc-phenolic compound

Time (day) /sample	0.5% FPECc	0.5% FPECc- 0.5% GTE	0.5% FPECc- 0.5% GSE
1	$0.11\pm0.06^{a,C}$	$1.05\pm0.16^{a,B}$	$2.57\pm0.37^{ab,A}$
3	$0.07\pm0.01^{ab,C}$	$1.03\pm0.07a^{b,B}$	$2.86\pm0.08^{a,A}$
5	$0.06\pm0.02^{b,C}$	$0.97\pm0.02^{ab,B}$	$2.59\pm0.23^{ab,A}$
7	$0.05\pm0.01^{b,C}$	$0.92\pm0.04^{b,B}$	$2.39\pm0.32^{b,A}$
14	$0.07\pm0.02^{ab,C}$	$0.78\pm0.02^{c,B}$	$2.28\pm0.09^{b,A}$

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples within the same storage time. Lower case letters are for values of the same sample in various time points)

Table B25. The data table for viscosity results determined for all pectin-phenolic compound emulsions

	Control	**0.5% GTE**	**0.5% GSE**	**0.5% CAT**	**1% CAT**
0.5% CPEC	$49.93 \pm 1.21^{a,A}$	$37.66 \pm 4.04^{a,B}$	$43.47 \pm 3.47^{a,B}$	$0.36 \pm 0.01^{a,C}$	$0.47 \pm 0.07^{b,C}$
0.5% FPECn	$41.63 \pm 6.82^{ab,AB}$	$46.39 \pm 6.39^{a,A}$	$33.79 \pm 5.47^{a,B}$	$0.87 \pm 0.60^{a,C}$	$1.68 \pm 0.01^{a,C}$
0.5% FPECc	$31.48 \pm 0.99^{b,B}$	$34.66 \pm 3.79^{a,B}$	$44.71 \pm 6.14^{a,A}$	$1.50 \pm 0.23^{a,C}$	$1.39 \pm 0.41^{a,C}$

(Values are given as mean ± standard deviation. Different letters indicate significantly different values at $P \leq 0.05$. The capital letters are for values of different samples of pectin. Lower case letters are for values of the same sample in various time points)