ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF TURKISH EXTRA VIRGIN OLIVE OIL FROM DIFFERENT VARIETIES

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ABSTRACT

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF TURKISH EXTRA VIRGIN OLIVE OIL FROM DIFFERENT VARIETIES

Olive oil is the main source of fat in Mediterranean diet. The major active components of olive oil include oleic acid, phenolic compounds and squalene which have different benefits such as cancer prevention, antimicrobial and antioxidant activities, and lowering the incidence of skin cancer, respectively. In recent years, the number of studies about the biochemical properties of different varieties of olive oils and their phenolic contents has dramatically increased.

In this study, Turkish EVOO from different varieties and their phenolic compounds were investigated in terms of their antimicrobial and antioxidant properties as well as refined olive, hazelnut and canola oils. Antimicrobial activities of EVOOs and ten phenolic compounds were tested against three foodborne pathogenic bacteria, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Enteritidis which are the foremost bacterial cause of the largest number of outbreaks, cases and deaths in the world. While all EVOOs showed bactericidal effect the phenolics demonstrated slight antimicrobial activity with percent inhibition between 0-25 of growth rate of bacteria. Moreover, refined olive, hazelnut and canola oils did not show any antimicrobial activity.

Finally, antioxidant activities of EVOOs, refined olive, hazelnut and canola oils were determined by β-carotene-linoleic acid model system and ABTS radical scavenging method. According to β-carotene-linoleic acid method, it was observed that antioxidant activities of oil samples varied between 21.19% and 64.54%. On the other hand, it was found that free radical scavenging activities varied between 1.21% and 21.97% as a result of ABTS method. These results were correlated with TPC values of oils.

ÖZET

FARKLI TÜRLERDEKİ TÜRK SIZMA ZEYTİNYAĞLARININ ANTİMİKROBİYAL VE ANTİOKSİDANT AKTİVİTELERİNİN ARAŞTIRILMASI

Zeytinyağı, Akdeniz diyetinin başta gelen yağ kaynağıdır. Oleik asit, fenolik bileşikler ve skualen, zeytinyağının en önemli aktif bileşenleridir. Bu maddeler sırasıyla kanserin önlenmesi, antimikrobiyal ve antioksidant aktivite, ve cilt kanserinin görülme sıklığını azaltması gibi çeşitli faydalara sahiptir.

Bu çalışmada, farklı türlerdeki Türk sızma zeytinyağları ve içeriğindeki fenolik bileşikleri antimikrobiyal ve antioksidant aktiviteleri açısından incelenmiştir. Dünyada en çok salgın, hastalık vakası ve ölüme neden olan üç gıda patojeni olan *Escherichia coli* O157:H7, *Listeria monocytogenes* ve *Salmonella* Enteritidis üzerine sızma yağlar ve on adet fenolik bileşiğin antimikrobiyal aktiviteleri incelenmiştir. Tüm sızma zeytinyağı örnekleri, bakterisidal etki gösterirken, fenolik bileşikler bakterilerin büyüme hızında %0-25 arası inhibisyon oranıyla çok düşük antimikrobiyal aktivite göstermiştir. Öte yandan, rafine zeytinyağı, fındık ve kanola yağları antimikrobiyal aktivite göstermemiştir.

Son olarak, sızma zeytinyağlarının, rafine zeytin, fındık ve kanola yağlarının antioksidant aktiviteleri β-karoten-linoleik asid model sistemi ve ABTS radikal temizleme metodlarıyla test edilmiştir. β-karoten-linoleik asid metodu sonucunda yağ örneklerinin %21.19 ile %64.54 arasında aktivite gösterdikleri bulunmuştur. Öte yandan, ABTS yöntemiyle yağların serbest radikal temizleme aktiviteleri %1.21 ile %21.97 arasında değişen değerlerde bulunmuştur. Tüm antioksidant sonuçları yağların toplam fenol içerikleriyle (TPC) ilişkilendirilmiştir.

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ABBREVIATIONS

ABTS	2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ATCC	American Type Culture Collection
a_w	Water activity
B. cereus	*Bacillus cereus*
BHA	Butylated hydroxyanisol
BHI	Brain Heart Infusion
CDC	Centers for Disease Control and Prevention
cfu	Colony forming unit
CHD	Coronary Heart Disease
Cin	Cinnamic acid
DAEC	Diffuse-adhering *E. coli*
DPPH	2,2- diphenyl-1-picrylhydrazyl
E. coli O157:H7	*Escherichia coli* O157:H7
EAEC	Enteroaggregative *E. coli*
EFSA	European Food Safety Authority
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinovasive *E. coli*
EPEC	Enterophatogenic *E coli*
ETEC	Enterotoxigenic *E. coli*
EVOO	Extra Virgin Olive Oil
FDA	Food and Drug Administration
FRSA	Free Radical Scavenging Activity
g	Gram
GA	Gallic acid
GSH	Glutathione
h	Hour
H. pyroli	*Helicobacter pylori*
HDL	High Density Lipoprotein
HUS	Hemorhagic Uremic Syndrome
IOOC	International Olive Oil Council

L	Linoleic acid
l	Liter
L. monocytogenes	*Listeria monocytogenes*
LB	Lauria-Bertani
LDL	Low Density Lipoprotein
Ln	Linolenic acid
min	Minute
MIC	Minimum Inhibitory Concentration
ml	Mililiter
µl	Microliter
O	Oleic acid
OD	Optical Density
ORAC	Oxygen Radical Absorbance Capacity
P	Palmitic acid
ppm	Parts per million
ROS	Reactive Oxygen Species
rpm	Revolutions per minute
S	Stearic acid
S. aureus	*Staphylococcus aureus*
S. Enteritidis	*Salmonella* Enteritidis
S. Typhimurium	*Salmonella typhimurium*
TPC	Total Phenolic Content
TSB	Tryptic Soy Broth
TSA	Tryptic Soy Agar
Tyr	Tyrosol
V	Vanillin
VA	Vanillic Acid
vol/vol	Volume per volume

CHAPTER 1

LITERATURE REVIEW

1.1. Food-borne Pathogenic Bacteria and Outbreaks

Consumption of contaminated food causes more than 200 diseases. Food-borne illnesses resulting from consumption of foods contaminated with pathogenic bacteria or viruses have been a major public health concern in the world (Mead, et al. 1999). The cells of enteropathogenic bacteria remain alive in the food and water during consumption. Viable cells, even if present in small numbers, have the potential to establish and multiply in the digestive tract to cause the illness (Ray 2003).

Transmission/contamination often occurs when microorganisms contaminate the food preparation areas and are allowed to multiply in food e.g. due to inadequate storage temperatures, inadequate cooking or cross contamination of ready-to-eat food. In addition, microorganisms may be transmitted to humans by direct contact with infected animals or fecally contaminated water, environments and foods (EFSA 2009).

The food-borne agents causing the most of the deaths are *Salmonella* (31%), *Listeria* (28%), *Toxoplasma* (21%), Norwalk-like viruses (7%), *Campylobacter* (5%), and *E. coli* O157:H7 (3%) (Mead, et al. 1999). In the US, the annual cost of food-borne diseases is estimated to be more than $20 billion. Costs in 1996 were estimated to be $20 to $37 billion for food-borne illnesses caused by seven pathogens: *Campylobacter jejuni, Clostridium perfringens, Escherichia coli* O157:H7*, Listeria monocytogenes, Salmonella* sp., *Staphylococcus aureus,* and *Toxoplasma gondii. Salmonella* sp. is the one causes the largest number of food-borne outbreaks, cases and deaths among all other food-borne pathogenic bacteria (Ray 2003).

Food Net of CDC's Emerging Infections Program has collected the data from 10 U.S. states, and reported that a total of 17,883 laboratory-confirmed cases of infection were recorded in 2007. The number of cases and incidences caused by food-borne pathogenic bacteria in 2007 in the USA alone were reported in Table 1.1.(CDC 2008-a).

On the other hand, many cases of food-borne illnesses are not reported because people usually do not seek medical care when they have illness (Mead, et al. 1999).

Table 1.1. The incidence of laboratory-confirmed bacterial infections in 2007 in USA (Source: CDC 2008-a)

Pathogen Bacteria	Number of Cases	Incidence per 100,000 Population
Salmonella	6,790	14.92
Campylobacter	5,818	12.79
Shigella	2,848	6.26
STEC O157	545	1.20
STEC non-O157	260	0.57
Yersinia	163	0.36
Listeria	122	0.27
Vibrio	108	0.24

In order to reduce health hazards and economic losses due to food-borne microorganisms, the use of natural products as antibacterial compounds seems to be an interesting way to control the presence of pathogenic bacteria and to extend the shelf life of the processed food.

1.1.1. *Escherichia coli* O157:H7

Escherichia coli (*E. coli*) is a Gram-negative, motile, non-sporulating, rod-shaped, facultative anaerobic bacterium (Ray 2003). *E. coli* is a common part of the normal micro flora in the intestinal tract of healthy human and warm-blooded animals. Most *E. coli* strains are harmless; however, some are pathogenic and cause diarrheal disease. In terms of their virulence mechanism, pathogenic *E. coli* strains are classified into six groups: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinovasive *E. coli* (EIEC), diffuse-adhering *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), and enterohemorrhagic *E. coli* (EHEC) (Doyle 2001). The most well-

known member of EHEC group is *E. coli* O157:H7 which was first identified as a food-borne pathogen in 1982 (Wells, et al. 1983, Duffy, et al. 2006, Riley, et al. 1983).

The optimum growth temperature range for *E. coli* is between 30°C and 42°C, and no growth occurs under 10°C (Bhunia 2008). An important property of *E. coli* is its ability to survive well in food at -20°C. In contrast to most food-borne pathogens, many strains of *E. coli* O157:H7 are resistant to acidic environments. The minimum pH for *E. coli* O157:H7 growth is 4.0 to 4.5 (Ray 2003). *E. coli* O157:H7 can grow in NaCl concentration of 66.5% (Glass, et al. 1992).

Some of the sources and reservoirs of *E. coli* O157:H7 including beef and lamb, lettuce, sprouts, fruit juices, vegetables, raw milk, and water have been implicated as vehicles of transmission (Duffy, et al. 2006, Ray 2003). Since *E. coli* O157:H7 grows in the intestines of mammals and is found in fecal material, it can spread through fecally contaminated water and vegetables irrigated with polluted water (Madigan, et al. 2002).

It has been reported that 10 to 100 cells are enough to cause disease in humans. After an incubation period of 3-4 days, bacteria cause watery diarrhea followed by abdominal cramping pain for 1-3 days (Karch, et al. 2005). If bacteria enter the blood stream, they spread and infect the other organs. When they infect the kidney, especially in children and the elderly, it leads to kidney failure which is called Hemolytic Uremic Syndrome (HUS) (Black 2004). From 1 to 14 days after exposure, symptoms can be seen (Coia 1998). However, the illness may last more than a few months and cause permanent damage or even death (Duffy, et al. 2006, Karch, et al. 2005). In addition, *E.coli* O157:H7 can survive in the contaminated environment for more than 10 months after the initial contamination; therefore, it is a major threat to human health (Karch, et al. 2005).

1.1.2. *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive, motile, non-sporulating, rod shaped, psychrotrophic and facultative anaerobic bacterium. They are 1–2 μm long and may exist as single or double cells. *L. monocytogenes* may form long chains in different growth conditions and temperatures (Bhunia 2008). The cells have endurance against

harsh conditions such as freezing, drying, high salt concentrations, and pH 5.0 and above (Ray 2003). Although *L. monocytogenes* can grow at temperatures between 0^{o}C to 45^{o}C, its growth is much slower at lower temperatures (Doyle 2001). Although the optimal growth temperature is ~37^{o}C for *L. monocytogenes,* it has ability grow at refrigeration temperatures (Ryser and Marth 2007). Most bacteria can not survive under 4^{o}C, however *L. monocytogenes* has ability to survive even at -7°C (Ramaswamy, et al. 2007). As a result, it may be transmitted via ready-to-eat foods that have been properly refrigerated. The pasteurization temperatures of either 71.7°C for 15 s or 62.8°C for 30 min can kill the cells. However, when they are inside the white blood cells, a temperature of 76.4°C to 77.8°C for 15 s is required (Ray 2003). *L. monocytogenes* has ability to survive and grow in foods having moderate to low pH values. It can initiate growth in the pH range of 4.4 - 9.6 (Doyle 2001). However, the incubation temperature and the type of the acid influence the growth of bacteria at acidic pH values (Ryser and Marth 2007). The water activity (a_w) value for the optimum growth of *L. monocytogenes* is about 0.97. On the other hand, it can multiply at a_w values as low as 0.90 in contrast to most food-borne pathogens (Doyle 2001, Gandhi and Chikindas 2007, Low and Donachie 2007). *L. monocytogenes* is a halotolerant organism due to its ability to survive at high salt concentrations from 10 to 12% NaCl. However, it can grow more efficiently in moderate concentrations such as 6.5% NaCl (Doyle 2001).

It has been identified that 13 serotypes of *L. monocytogenes* which can cause disease. However, the most of human isolates belong to three serotypes which are 1/2a, 1/2b and 4b. About 50% of sporadic human cases in the world and all major outbreaks in Europe and North America arose from serotype 4b. (Doyle 2001). In addition, it has been reported that serotypes 1/2a, 1/2c, 1/2b, and 4b accounted for over 98% of the human listeriosis cases (Liu, et al. 2006).

L. monocytogenes isolates can be grouped into three major genetic divisions as lineages I, II and III. Lineage I predominantly includes serotypes 1/2b, 3b, 3c, and 4b, while lineage II primarily includes serotypes 1/2a, 1/2c, and 3a. *L. monocytogenes* lineage III, which appears to be predominantly associated with animals, is less common. Lineage I often comprises serotypes that are related with endemic human listeriosis. Lineage II also includes serotypes which are frequently associated with human disease; on the other hand, lineage III has been associated with only a few human listeriosis isolates (Liu, et al. 2006). Moreover, it has been reported that lineage I members may

represent a human host adapted lineage, while lineage II members may represent an environmentally adapted lineage (Nightingale, et al. 2005).

L. monocytogenes has the ability to attach to a wide variety of food contact surfaces and form biofilms (Ryser and Marth 2007). They are found widely in soil, water sewage, silage, plants, and in the intestinal tract of domestic animals (sheep, cattle, goat, etc.) (Bhunia 2008, Ryser and Marth 2007). The reported transmission vehicles of *L. monocytogenes* are raw and pasteurized milk, cheese, ice cream, poultry, raw meats, raw fruits and vegetables, raw and smoked fish and fermented raw meat sausages (FDA 2007-a). The first confirmed food-borne listeriosis outbreak occurred in 1981 in Nova Scotia, Canada which was caused from consumption of contaminated coleslaw (Doyle 2001).

L. monocytogenes is one of the most lethal food-borne pathogens. Although there are only about 2500 cases of acute listeriosis each year, about 500 cases end in death. Nearly all patients require hospitalization (Ryser and Marth 2007). The infective dose is unknown because it is strain and host dependent. In particular, *L. monocytogenes* is potentially fatal to newborns, the elderly and individuals with immune-deficiency such as AIDS, cancer, organ transplant and diabete patients (Low and Donachie 1997). Generally, symptoms appear 4 to 7 days after ingestion and include mild flu-like symptoms with fever, abdominal cramps, and diarrhea (Ray 2003). In addition, manifestations of listeriosis include septicemia, meningitis, encephalitis, and stillbirth. Recently, in August 2008, the worst epidemic listeriosis in the world happened in Canada. Among 43 confirmed cases of listeriosis, at least 16 Canadian was killed from contaminated cold cut which produced in a Toronto meat processing plant (Attaran, et al. 2008).

1.1.3. *Salmonella* Enteritidis

In the nomenclature of the genus *Salmonella*, there are several problems. One of these problems is that serovars of *Salmonella* were considered as species before 1970s. After molecular analysis of all, it was understood that typical Salmonellae were closely related and might be considered as a single species. In the nomenclature, the first letter

of serovar name is now capitalized and not italicized. For example, *Salmonella enterica* subsp. *enterica* serovar Enteritidis is used in place of *Salmonella enteritidis* for the first citation. Then, *Salmonella* serovar Enteritidis or *Salmonella* Enteritidis is used in subsequent references (Heyndrickx, et al. 2005).

Salmonella is a Gram-negative, non-sporulating, facultative anaerobic, motile, rod-shaped bacterium (Doyle 2001, Ray 2003). Their optimum growth temperature is around 37°C, but they generally can grow in a temperature range of 5°C to 46°C (Ray 2003). Although their optimal growth pH is around 6.5 to 7.5, they can grow at pH values ranging from 4.5 to 9.5. *Salmonella* spp. can not grow at foods which have a_w values of lower than 0.94. The growth of *Salmonella* spp. is generally inhibited in the presence of 3% NaCl. However, bacterial salt tolerance increases when the temperature is in the range of 10°C to 30°C (Doyle 2001). Moreover, although antibacterial activity of organic acids is reduced in the presence of salt, the growth of serovar Enteritidis is induced in broth medium (pH 5) which contains NaCl or KCl at low concentrations and acidified with acetic acid (Radford 1995).

Pasteurization is a good method for killing *Salmonella* spp. They are also sensitive to low pH values (4.5 and below), and especially in combination with an a_w of 0.94. On the other hand, the cells can survive in frozen and dried state for a long time. An important point is that they can grow in various foods and the accepted quality of food can not be affected from that (Ray 2003).

Newborns, infants, the elderly, and immuno-compromised individuals are more susceptible to *Salmonella* infections than healthy adults. The ingestion of only a few *Salmonella* cells can cause disease. Human infections with *Salmonella* commonly give rise to enterocolitis, which appears 8 to 72 h after ingestion (Doyle 2001). Generally, the disease lasts 4 to 7 days, and most of the patients can recover without antibiotic treatment (CDC 2008-b).

Foremost sources of salmonellosis outbreaks are raw meat, poultry, eggs, milk and dairy products, water, fish, fresh consumed tomato and lettuce, sauces and salad dressing, peanut butter, cocoa, and chocolate (FDA 2007, Black 2004, CDC 2008b). Recently, by the use of green fluorescent protein marked bacteria, it has been observed that *Salmonella* can enter the cells of the plant and multiplies in the host cells (Schikora, et al. 2008). Therefore, it is impossible to protect form *Salmonella* infection only by washing, if the vegetables contain the organism in their cells or tissues.

Salmonellosis is the most frequently occurring food-borne disease in the world and the annual number of salmonellosis cases is estimated as 4 million in the U.S. (FDA 2007). According to the latest report of EFSA about the food-borne outbreaks, *Salmonella* was the most commonly reported cause of outbreaks in EU, as in previous years. A total of 2,201 outbreaks were verified in 22 member states of EFSA and *Salmonella* caused 590 of these outbreaks which affected 8,922 people, resulted in 1,773 hospitalizations and ten deaths. Also, it was reported that the most common serovar was *S.* Enteritidis and eggs/egg products were the most frequent sources of these outbreaks (EFSA 2009).

In the prevention of salmonellosis, the maintenance of clean water and food supplies is important. The organisms can not be entirely removed since poultry and other animals serve as reservoirs, and no effective vaccine is available (Black 2004).

1.2. Mediterranean Diet and Olive Oil

1.2.1. Mediterranean Diet

'Mediterranean diet' is not a homogenous nutritional model because Mediterranean dietary patterns change due to different cultures, traditions, religions and income level. As a result, there are a wide variety of the dietary patterns within the Mediterranean region (Simopoulos 2007). Mediterranean diet can be defined as a diet that has a high consumption of olive oil, vegetables, legumes, fruits, cereals and, fish, with regular but moderate ethanol intake, mostly during meals, and low consumption of meat and dairy products (Trichopoulou and Dilis 2007).

Mediterranean diet is a healthy dietary model to achieve healthy aging and to prevent the most important diseases such as cancer, coronary heart disease (CHD). Health benefits of the Mediterranean diet is related to (i) the consumption of lesser amounts of saturated fats; (ii) the consumption of greater amounts of monounsaturated fatty acids (MUFAs) due to olive oil, and (iii) the intake of omega-3 fatty acids from fish, wild plants, nuts and legumes . Benefits of this diet have also been associated with

bioactive compounds such as vitamins and natural antioxidants. Such compounds are found naturally in fresh fruits, vegetables, nuts, seeds, whole grain products, herbal teas, wine, olive and olive oil (Simpoulos 2007).

Epidemiological studies have shown that some certain diseases such as artherosclerosis, cardiovascular heart diseases and certain kinds of cancer were occurred in Mediterranean region not as often as in other regions (Tripoli, et al. 2005). It has been found that the risk for most epithelial cancers decreased with increasing vegetable and fruit consumption. Mediterranean diet has a protective effect on breast, female genital tract, urinary tract and a few other epithelial neoplasms (Vecchia 2004).

1.2.2. Olive oil

Olive oil is a major component of the diet of the Mediterranean countries such as Spain, Italy, Greece, Tunisia, Turkey, Syria and Portugal. For the people living in this region, olive oil is the main source of fat in their cuisines. In the past few years, olive oil has also become more popular among consumers in Northern Europe, China, Japan, the US and Canada (Gunstone 2002).

Olive oil is the vegetable oil obtained from the fruit of the olive tree (*Olea europea sative)* by mechanical extraction (IOOC 2008). Types of olive oil are as follows;

(i) Virgin olive oil is obtained by mechanical or other physical means under conditions, particularly thermal, which do not lead to changing of the oil. Virgin olive oil is suitable for consumption in natural state (IOOC 2008).

(ii) Extra virgin olive oil is a virgin olive oil which has a free acidity, expressed as oleic acid, of not more than 0.8 grams per 100 grams (IOOC 2008).

(iii) Refined olive oil is obtained from virgin olive oil by refining methods, which make it suitable for consumption in the natural state and do not lead to alteration in the initial glyceridic structure (IOOC 2008).

(iv) Refined olive-pomace oil is the oil obtained from olive pomace by extraction by using solvents and made edible by refining processes not altering the initial glyceridic structure (IOOC 2008).

In contrast to other edible oils with a similar fatty composition, particularly, sunflower, hazelnut, soybean and rapeseed, canola oils, virgin olive oil is a natural juice. The seed oils must be refined before consumption and this process changes their chemical compositions. Virgin olive oil is a source of healthy unsaturated fatty acids and numerous micronutrients, especially antioxidants, such as phenolic compounds, carotenes and vitamin E (Waterman and Lockwood 2007, Jaen 2005).

Virgin olive oil plays role in the prevention of many diseases, such as cancer, coronary heart disease (CHD), and rheumatoid arthritis. Virgin olive oil has a protective effect from cancer due to its ability to prevent the DNA damage by oxidation. Some of the compounds present in virgin olive oil such as oleuropein, hydroxytyrosol, and tyrosol can also act as potent antioxidants. For example, it has been found that hydroxytyrosol and oleuropein have anticancer effect by prevention of DNA damage, and they inhibit the LDL oxidation (Waterman and Lockwood 2007). Several of the components of olive oil can have anticancer properties such as oleic acid, flavonoids, vitamin E, squalene, caffeic acid and hydroxytyrosol (Pamplonai, et al. 2002, Panza, et al. 2004, Jaen 2005).

Vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients was investigated by Visioli et al. (2005). Twenty two patients were administered 40 ml/day of either extra virgin (rich in phenols), or refined (poor in phenols) olive oils. Consumption was related with affirmative changes in circulating markers of cardiovascular conditions. The researchers concluded that phenol rich olive oil in the diet can positively modify surrogate biological markers for cardiovascular diseases.

1.3. Composition of Olive Oil

Olive oil is a complex mixture consisting of two main groups of substances: (i) saponifiables, the major components, which represent nearly 99% of the chemical composition, such as triacylglycerols, partial glycerides, esters of fatty acids or free fatty acids and phosphatides; and (ii) unsaponifiables, the minor components, which represent only about 1% of all olive oil composition, such as phytosterols, tocopherols,

hydrocarbons, pigments, phenolic compounds, flavonoids or volatile compounds (Tripoli, et al. 2005, Göğüş, et al. 2009) (Table 1.2.).

Table 1.2. Components of olive oil
(Source: Göğüş, et al. 2009)

Major Components (99%) (Saponifiable Compounds)	Minor Components (1%) (Unsaponifiable Compounds)
❖ Fatty acids ❖ Triacylglycerols	❖ Hydrocarbones ❖ Sterols ❖ Tocopherols ❖ Volatile compounds ❖ Fatty Alcohol, Waxes and Diterpene Alcohols ❖ Mono and diacylglycerols ❖ Pigments ❖ Phenolic compounds

1.3.1. Major Components

1.3.1.1. Triacylglycerols and Fatty Acids

Triacylglycerol content of olive oil is composed of OOO (40-59%), POO (12-20%), OOL (12.5-20%), POL (5.5-7%), SOO (3-7%) and POP, POS, OLnL, LOL, OLnO, PLL, PLnO and LLL, LnLO, LnOP, PLP, SOP, EOO in very small amounts. These three letter symbols stand for all the isomeric triacylglycerols containing the three acyl groups indicated where P=palmitic, O=oleic, S=stearic, L=linoleic and Ln=linolenic acid (Gunstone 2002).

The fatty acid content of olive oil is comprised of myristic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, eicosanoic acid, gadoleic acid, behenic acid, lignoceric acid (IOOC 2008).

1.3.2. Minor Components

The minor constituents of olive oil can be classified into two major groups: those which are fatty acid derivatives and the compounds that have different chemical structure. The first group is composed of phospholipids, waxes, and sterylesters, while the second group contains hydrocarbons, aliphatic alcohols, free sterols, tocopherols, pigments, and polar phenols such as hydroxytyrosol (Simopoulos 2007).

1.3.2.1. Hydrocarbons

Squalene is a triterpene hydrocarbon and a major intermediate compound in the biosynthesis of cholesterol (Simopoulos 2007). It is found in both plants and animals, but it exists in different amounts. For example, while olive oil is comprised of approximately 0.7% squalene, other foods and oils typically contain it in the range of 0.002-0.03% (Waterman and Lockqood 2007). It has been reported that squalene accounts for more than 50% of the unsaponifiable content of olive oil and its level in the oil can be between 200 and 7500 mg/kg (Boskou 2009). Researchers showed that squalene is a causal factor for the lower incidence of cancer, especially skin cancer, in Mediterranean countries (Waterman and Lockqood 2007, Jean 2005).

1.3.2.2. Sterols

Sterols are important in terms of oil quality. Sterols in olive oil can be classified into four groups as common sterols, 4α-methylsterols, triterpene alcohols and triterpene dialcohols. The total level of them is about 1000 mg/kg in virgin olive oils (Boskou 2006). Phytosterols are very important for human nutrition. They have a cholesterol lowering effect (Simpoulos 2007). Several studies have shown that phytosterols significantly reduced the level of total cholesterol and LDL in the plasma (Moghadasian

2000). In contrast, in a research about the effect of vegetable oils on LDL, an olive oil-rich diet resulted in higher concentration of LDL in the blood than rapeseed oil and sunflower oil rich diets. It has been suggested that this difference might be associated with difference in squalene and phytosterol concentrations of these oils (Anetta, et al. 2002).

1.3.2.3. Tocopherols

Tocopherols are important fat-soluble vitamins. They have a great contribution to the stability of oils and also play a beneficial biological role as free radical quenchers *in vivo*. Nutritional benefits of olive oil are due to its fatty acid composition and the content of α-tocopherol and other natural antioxidants. The α-tocopherol acts as a free radical trapping agent and also as a singlet oxygen quencher. Additionally, β-carotene also contributes to protection against photo-oxidation by the help of its antioxidant property (Simopoulos 2007, Banerjee 2008). The concentration of α-tocopherol in extra virgin olive oil is higher than 200mg/kg (Boskou 2009).

1.3.2.4. Volatile and Aroma Compounds

Volatile and aroma compounds contribute to the aroma of virgin olive oil. More than 250 constituents have been identified. These are mainly hydrocarbons, alcohols, aldehydes, ketones, acids, esters, ethers, and furan derivatives (Simpoulos 2007, Boskou 2006).

1.3.2.5. Fatty Alcohol, Waxes and Diterpen Alcohols

Aliphatic and aromatic alcohols are found as the free and esterified form in olive oil. The most importants are fatty alcohols and diterpene alcohols. It has been reported that alkanols and alkenols with less than ten carbon atoms like benzyl alcohol and 2-phenyl ethanol are constituents of the volatile fraction of olive oil. In terms of chemical structures, fatty alcohols are mainly linear saturated alcohols which have more than 20 carbon atoms, such as docosanol, tetracosanol and hexacosanol. Their levels in virgin olive oil are not usually higher than 250 mg/kg. Waxes are used to differentiate various olive oil types. The level of waxes is lower than 150 mg/kg in virgin olive oil, while this level increases up to higher than 2000mg/kg in refined olive oil (Simpoulos 2007, Boskou 2006).

1.3.2.6. Mono and Diacylglycerols

In olive oil, monoacyl- and diacylglycerols are always found in small quantities. The presence of partial glycerides is as a result of either incomplete biosynthesis of triacylglycerols or hydrolysis. In virgin olive oil, diacylglycerols is present in the range of 1–2.8%, while monoacylglycerols are found in much smaller amounts less than 0.25%. During storage, 1, 2-diacylglycerols change to the more stable diacylglycerols by isomerization. This change is used as a good characteristic for the age and quality of the oil (Kiosseoglou and Kouzounas 1993, Simopoulos 2007, Perez, et al. 2001, Spyros, et al. 2004).

1.3.2.7. Pigments

The presence of chlorophylls and carotenoids gives the green and yellow hue of the virgin olive oil (Boskou 2006). Chlorophyll content may vary between 10 and 30

mg/kg. When the light is absent, chlorophyll may act as a weak antioxidant. In contrast, in the presence of light, they act as a strong oxidation promoter. β-carotene and lutein are the main carotenoids of olive oil. Their total concentration may vary between 1 and 20 mg/kg. It is important that carotenoids play role in protection of oil from photo-oxidation by the help of its ability to quench singlet oxygen (Gunston 2002).

1.3.2.8. Phenolic Compounds

Phenolic compounds are a class of chemical compounds consisting of one or more hydroxyl groups attached directly to an aromatic ring. Polyphenols are compounds that have more than one hydroxyl group attached to one or more benzene rings. These compounds are characteristic to plants and they are usually found as esters or glycosides rather than as free forms (Vermerris and Nicholson 2006).

Phenolic compounds are classified as secondary metabolites rather than primary metabolites. They are synthesized through the shikimate pathway and phenylpropanoid metabolism. They are found in all parts of the plant but their nature and concentration vary significantly among the different tissues. The most of them are accumulated in the vacuole of the cell while some of them are stored either in the cell walls or in extracellular cuticles (Ryan, et al. 2002).

Oxidation of phenolic compounds can result in the browning of tissues. Oxidation can also result in the formation of metabolites that are toxic to animals and plants, and that can be explained as spoilage of foods in processing. On the other hand, the toxic compounds formed from the oxidation of phenolics can inhibit the growth of pathogenic microorganisms. In addition, certain phenols are used as antioxidants to prevent the oxidation of fatty acids (Vermerris and Nicholson 2006).

According to the structure of their carbon skeleton, polyphenols are classified as simple phenols and phenolic acids, flavonoids, stilbenes and lignans, and other ill-defined phenolic polymers. Flavonoids are the most abundant polyphenols in our diet; on the other hand, stilbenes are not common in food plants. Flavonoids can be divided into several classes according to the degree of oxidation of oxygen heterocycles: flavones, flavonols, flavanols, isoflavones, flavanones, anthocyanins and

proanthocyanidins. The subsequent abundant type of polyphenols is phenolic acids, in particular caffeic acid. Other dietary polyphenols are ill-defined chemicals which usually arise from food processing such as fermentation, storage, cooking and other processes. These are the main polyphenols which are found in black tea and wine (Simopoulos 2007).

The major phenolic compounds found in olive oil are, in alphabetic order, 1-acetoxypinoresinol, apigenin, caffeic acid, cinnamic acid, o- and p-coumaric acids, ferulic acid, gallic acid, p-hydroxybenzoic acid, hydroxytyrosol, luteolin, oleuropein, pinoresinol, protocatechuic acid, sinapic acid, syringic acid, tyrosol, vanillic acid, and vanillin (Boskou 2006). Molecular structures of these compounds are shown in Figure 1.1.

The quality of virgin olive oil is affected by the presence of phenolic compounds in olive fruits, as these compounds are partly responsible for the stability and sensory characteristics (Soni, et al. 2006). Extra virgin olive oil is rich in phenolic compounds. On the other hand, virgin olive oil has higher content of phenols, o-diphenols, hydroxytyrosol and tyrosol aglycones, and tocopherols. Oils obtained through centrifugation (second extraction) have a lower phenolic content, probably because this process involves the use of large amount of hot water that remove a significant amounts of the phenolic compounds (Tripoli, et al 2005).

The major phenolic constituent of olive and olive oil is composed of tyrosol and hydroxytyrosol in their various forms (Boskou 2006). They are derived from the hydrolysis of oleuropein (Tripoli, et al. 2005). The content of phenolic compounds in olives and olive oil depends on the cultivars, climate, preparation and storage of the oil, and the ripeness state of the fruit at the time of harvest (Soni, et al. 2006). In nature, the concentration of hydroxytyrosol and tyrosol increases as the fruits ripen while the total amount of phenolic compounds and α-tocopherol decreases (Tripoli, et al. 2005).

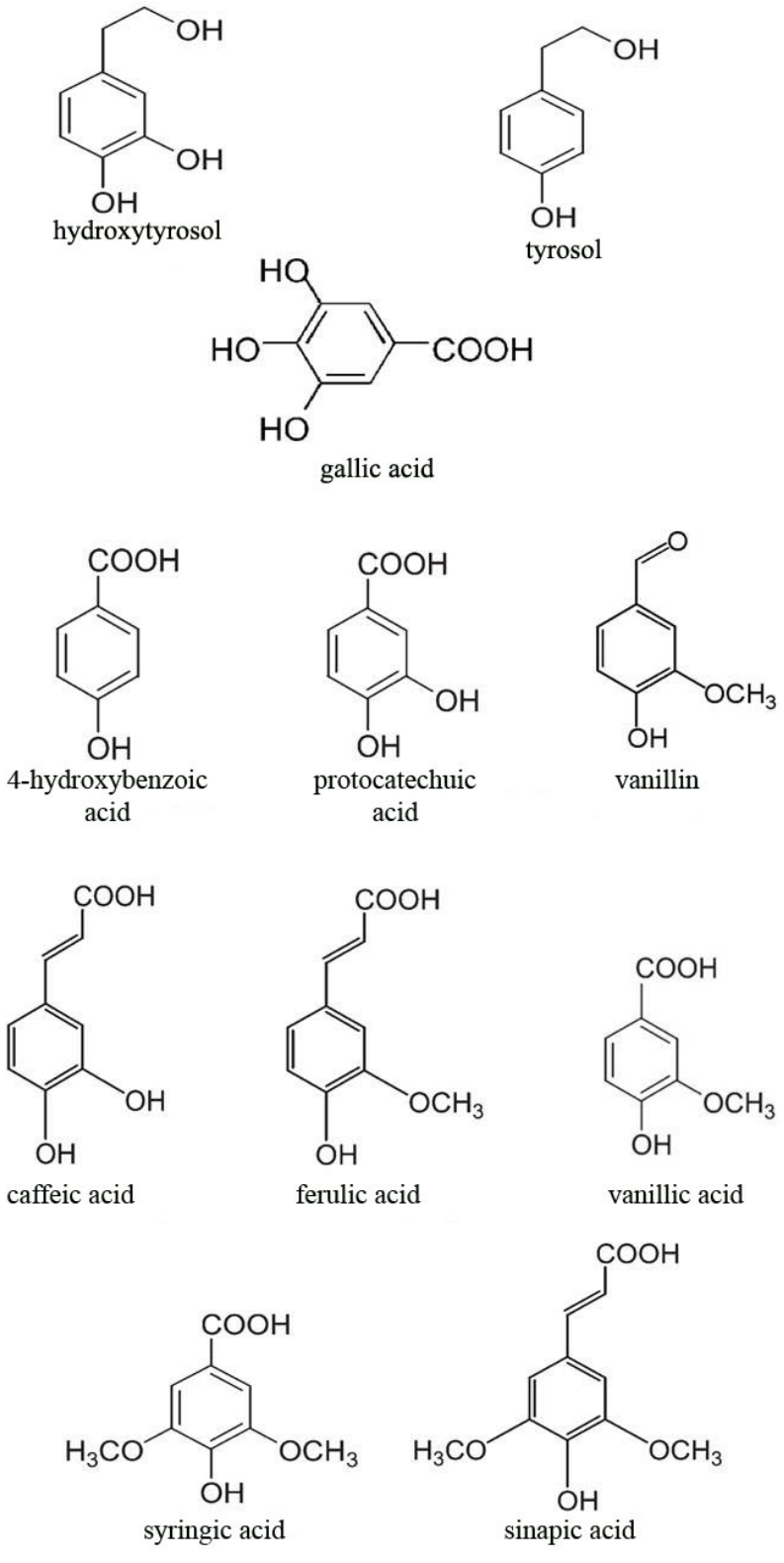

Figure 1.1. Molecular structures of hydroxytyrosol, tyrosol, gallic, 4-hydroxybenzoic, protocatechuic acids, vanillin, caffeic, ferulic, vanillic, syringic, synaptic, cinnamic, p- and o-coumaric acids, pinoresinol and acetoxypinoresinol (Source: Boskou 2009)

(Cont. on next page)

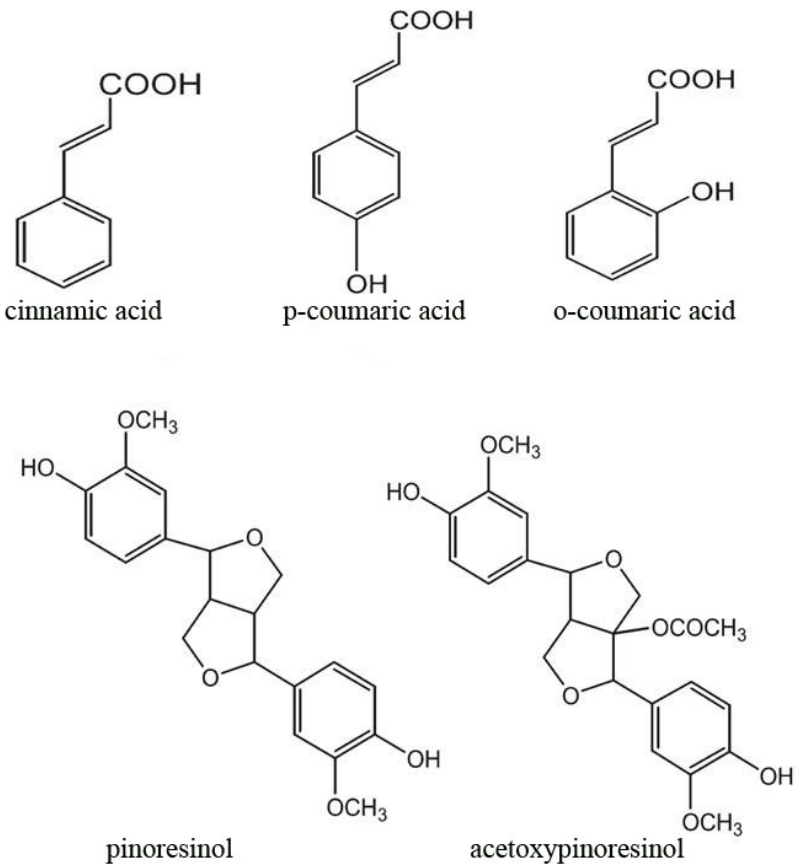

Figure 1.1. (cont.)

Hydroxytyrosol is one of the main phenolic compounds in olives, virgin oil and waste water obtained during the production of olive oil. In fresh virgin oil, hydroxytyrosol mostly occurs as esterified with elenolic acid to form oleouropein aglycone. On the other hand, as time passed, the concentration of the non-esterified forms increaces as a result of hydrolytic reactions (Tripoli, et al. 2005, Soni, et al. 2006). The presence of hydroxytyrosol has also been identified and quantified in Italian wines (Di Tommaso et al., 1998). Pure hydroxytyrosol is a clear, colorless, tasteless liquid and can be hydrosoluble or liposoluble. It greatly contributes to the shelf life of olive oil due to its ability to prevent it from oxidation (Soni, et al. 2006). The reported concentration of hydroxytyrosol is about 14.42 mg/kg in extra virgin olive oil and is about 1.74 mg/kg in refined olive oil (Tuck and Hayball 2002). Another major phenolic compound of olive oil is tyrosol that has a faint sweet fruity-floral odor and a very weak sweet taste (Soni, et al. 2006). The structure of tyrosol is identical with hydroxytyrosol except that there is an extra hydroxyl group in the *meta* position of hydroxytyrosol

structure (Figure 1.1.). The reported concentration of tyrosol is about 27.45 mg/kg in extra virgin olive oil while this level is much lower in refined olive oil (~2.98 mg/kg) (Tuck and Hayball 2002).

1.4. Biological Properties of Phenolic Compounds

1.4.1. Antimicrobial Properties of Phenolic Compounds

Phenolic compounds disrupt cell membranes, denature proteins, and inactivate enzymes. They are used as surface disinfectants and to destroy discarded cultures because organic materials do not reduce their antimicrobial actions (Black 2004).

It has been demonstrated that phenolic compounds present in olive products, such as oleuropein, hydroxytyrosol and aliphatic aldehydes, have ability to inhibit or delay the growth of a range of bacteria and fungi (Pereira, et al. 2006). Hydroxytyrosol has been shown to inhibit or retard the growth of a range of organisms such as fungi and human pathogens (Soni, et al. 2006). In another study, the antimicrobial activities of hydroxytyrosol and oleuropein against several human intestinal or respiratory tract pathogens were studied. It has been found that the broad antimicrobial activity of hydroxytyrosol against the several ATCC strains and clinical isolates with the minimum inhibitory concentration (MIC) values of 0.24 - 7.85 µg/ml and 0.97 - 31.25 µg/ml, respectively. It was also suggested that hydroxytyrosol might be useful in the antimicrobial treatment of intestinal or respiratory tract infections in humans (Bisignano, et al. 1999).

In another study on antimicrobial effect of some olive phenolic compounds, 4-hydroxybenzoic, vanillic, syringic, o- and p- coumaric, caffeic, ferulic and 4-hydroxyphenylacetic acids and tyrosol were tested against 4 microorganism (*Staphylococcus aureus, Bacillus cereus, E. coli O157:H7, Salmonella typhimurium*) by agar dilution technique (Tunçel and Nergiz 1993). The minimum inhibitory concentrations of tested phenolic compounds from this study are given in Table 1.3.

Table 1.3. Minimum inhibitory concentrations (MIC) (μg/ml) of some phenolic compounds (Source: Tunçel and Nergiz 1993)

Phenolic Compound	***S. aureus***	***B. cereus***	***E. coli* O157:H7**	***S. Typhimurium***
4-hydroxybenzoic acid	550	400	550	400
Vanillic acid	550	400	550	400
Syringic acid	600	400	550	400
o-coumaric acid	>600	400	450	400
p-coumaric acid	>600	400	450	400
Caffeic acid	400	350	350	350
Ferulic acid	600	400	450	400
Tyrosol	600	400	600	400
4-hydroxyphenylacetic acid	>600	400	600	400

Medina et al. (2006) determined the phenolic compound profiles of olive oils and other plant oils (sunflower, corn, rape-seed, soybean and cotton oils). Further, the profiles were correlated with the antimicrobial activities of these oils against 14 bacteria, including *L. monocytogenes, E. coli* and *S. enterica.* In addition, HPLC fractions of virgin olive oil were also tested against *L. monocytogenes.* According to the results of the bactericidal effects of all edible vegetable oils and the virgin olive oils against *E. coli* and *S. enterica*, virgin olive oil showed the highest activity, while the refined oil samples, which lost their phenolic compounds during the refining process, showed the lowest activity.

In a recent study, it has been found that virgin olive oil has bactericidal action against *Helicobacter pylori*, the primary cause of gastric ulcers and linked to gastric cancers. It has been concluded that phenols inhibited bacterial growth at low concentrations and were stable for several hours in the highly acidic environment of the stomach. It was found that the secoiridoid aglycones, especially the dialdehydic form of decarboxymethyl ligstroside, have the greatest antibacterial activity against *H. pylori* and are not hydrolyzed in the stomach. If hydrolysis occurs, it produces the less active hydroxytyrosol and tyrosol. Moreover, it was suggest that as the concentration of

phenolics needed to kill *H. pylori* is higher than that for antibiotics, the virgin olive oil should be considered as preventive rather than a treatment agent (Romero, et al. 2007).

1.4. 2. Antioxidant Properties of Phenolic Compounds

The 'reactive oxygen species' (ROS), which are continuously formed as a result of normal metabolic processes, can cause to the development of degenerative diseases in human such as atherosclerosis, cancer, diabetes, rheumatoid arthritis and inflammatory diseases. Exogenous antioxidants are important because they have two main functions which are prevention of food oxidation, in particular lipid peroxidation, and protection of organism against degenerative diseases. The most important dietary antioxidants are certain vitamins such as ascorbic acid, tocopherols, carotenes and phenolic compounds (Tripoli, et al. 2005, Banerjee 2008).

Phenolic compounds can act as antioxidants in various ways. In oxidative systems using transition metals such as Fe and Cu, phenolics can chelate metallic ions. This can prevent their involvement in Fenton reactions which can generate high concentrations of hydroxyl radicals. The most important antioxidant activity of phenolics is related to the free radical-scavenging ability, by breaking the chain of reactions triggered by free radicals (Baerjee 2008).

The antioxidant properties of the o-diphenols are due to their ability to form intramolecular hydrogen bonds between the hydroxyl group and the phenoxylic radicals (Visioli and Galli 1998). The number of –OH groups and their positions on the ring are important for both flavonoids and phenols. In other studies about the flavonoids, it has been proved that the degree of antioxidant activity is correlated with the number of hydroxyl groups (Rice-Evans, et al. 1996, Cao, et al. 1997). From the study of the resonance structures formed during the oxidation processes, it has been found that the ortho- and para-substitutes of the radicals are more stable than the meta-substitute molecules (Finotti and Majo 2003). In particular, ortho-diphenolic substitution gives higher antioxidant ability, while a single hydroxyl substitution, as in tyrosol, does not show any activity (Tripoli, et al. 2005).

There is a relation between phenolic compound content and oxidative stability of olive oil. A number of studies were done on determination of phenolic compound profiles and their oxidative stability comparisons of different olive oil samples (Blekas, et al. 2002, Haddada, et al. 2008). It has been reported that high polyphenol content is beneficial for the shelf life of the oil due to the correlation of stability and total phenol contents. In particular, it has been found that hydroxytyrosol and mainly aglycone forms of oleuropein contribute to the antioxidant effect of olive oil (Simopoulos 2007).

To evaluate whether olive oils high in phenolic compounds influence the oxidative/antioxidative status in humans, a research was carried out on healthy men participants by giving olive oil that was either low, or moderate, or high phenolic content. The results showed that the short term consumption of olive oils decreased plasma oxidized LDL, 8-oxo-dG in mitochondrial DNA and urine, malondialdehyde in urine, and increased HDL cholesterol and glutathione peroxidase activity in a dose-dependent manner with phenolic content of the olive oil administered. Phenolic compounds accumulate in plasma and urine after short term consumption and modulate the oxidative/antioxidative status in the human body (Weinbrenner, et al. 2004).

In *in vitro* and *ex vivo* models, olive oil phenolics have shown to have antioxidant properties, higher than that of vitamin E, on lipids and DNA oxidation (Fito, et al. 2007, Masella et al. 2004). To compare the protective effects of tyrosol and hydroxytyrosol, the oxidation of LDL was studied by means of J774A.1 (macrophages). For this purpose, intracellular ROS and Glutathione (GSH) content, and activities and expressions of GSH-related enzymes were evaluated. It was found that tyrosol was effective in inhibiting 30% of ROS production. Although tyrosol has a weak antioxidant activity, it was effective in preserving cellular defenses, this might be by accumulation in cell. Their findings show that consumption of olive oil prevents cardiovascular diseases (Benedetto, et al. 2007).

In a research on caffeic acid, it has been suggested that caffeic acid could have cytoportective effects on endothelial cells. This property was correlated not only with its action as an antioxidant agent but also with its ability to block the increase of the concentration of intracellular Ca^{+2} in response to lipoprotein oxidation. The ability of polyphenolic compounds to react with metal ions could make them pro-oxidant (Vieira, et al. 1998).

1.4.3. Other Biological Benefits of Phenolic Compounds

Various biological benefits of the phenolic compounds in olive oil have been reported. In particular, it has been found out that olive oil phenols inhibit platelet aggregation, reduce pro-inflammatory molecule formation such as thromboxane B_2 and leucotriene B_4, inhibit the use of oxygen in human neutrophils, increase nitric oxide (NO) production by the macrophages of rats exposed to endotoxin (Tripoli, et al. 2005).

Phenols are believed to act in the blood vessels to prevent LDL oxidation and in tissues to protect against DNA damage (Visioli, et al. 2002). It has been showed that short-term consumption of olive oils decreased the level of plasma oxidized LDL (oxLDL), and increased the level of HDL cholesterol and glutathione peroxidase activity, in a dose-dependent manner with the phenolic content of the olive oil administered (Weinbrenner, et al. 2004).

In vitro studies have demonstrated hydroxytyrosol and oleuropein are capable of inhibiting production of isoprostanes which is a marker of LDL oxidation. It has been suggested that phenols present in olive oil may act synergistically with these constituents to prevent LDL oxidation (Salami, et al. 1995).

They are also able to prevent the endothelial dysfunction by decreasing the expression of cell adhesion molecules, and increasing NO production and inducible NO synthesis by quenching vascular endothelium intracellular free radicals (Fito, et al. 2007). Also, it has been demonstrated that phenolic compounds can inhibit platelet-induced aggregation and enhance the mRNA transcription of glutathione peroxidase which is an antioxidant enzyme (Fito, et al. 2007, Masella, et al. 2004).

In another investigation about the benefits of hydroxytyrosol on human health (Manna, et al. 1999), intact human red blood cells exposed to H_2O_2 *in vitro* were used to test the ability of the olive oil hydroxytyrosol to prevent oxidative hemolysis and membrane lipid peroxidation. As a result, a reduced hemolysis was observed in cells pretreated with micromolar concentration of hydroxytyrosol. This result indicates that this phenolic compound effectively protects red blood cells against ROS-induced cytotoxicity. From a research about examining individual phenolic compounds, it has been found that hydroxytyrosol is capable of protecting cells from H_2O_2 damage and

DNA from peroxynitrite-induced damage, blocking cell cycle progression at the G1 phase, and inducing apoptosis (Fabiani, et al. 2002).

CHAPTER 2

INTRODUCTION

One of the most well-known and important characteristic of the Mediterranean diet is the presence of virgin olive oil as the principal source of fat. While the seed oils, such as sunflower, hazelnut, rapeseed canola oil, and soybean, must be refined before the consumption, virgin olive oil is a natural juice and has numerous healthy components such as fatty acids, squalene, carotenes, vitamin E and phenolic compounds.

The main phenolic compounds present in virgin olive oil are tyrosol, hydroxytyrosol and its secoroids and conjugate forms, and lignans. Researchers showed that phenolic compounds play a role in prevention from certain diseases such as cardiovascular heart diseases and cancers (Visioli, et al. 2005, Waterman and Lockwood 2007). In addition to the health benefits, phenolic compounds are important in terms of virgin olive oil quality because of their contribution to oil flavor and aroma. They also protect the olive oil from oxidation by the help of their antioxidant properties (Haddada, et al. 2008, Blekas, et al. 2002). In addition to their antioxidant properties, it has been found that phenols also have antimicrobial activity. In a recent research, antimicrobial activities of different edible vegetable oils were correlated with their phenolic contents (Medina, et al. 2006).

The objective of this study was to determine the antimicrobial and antioxidant activities of Turkish EVOOs from different varieties. Antimicrobial activities were investigated against three food-borne pathogenic bacteria, *E. coli* O157:H7, *L. monocytogenes* and *S.* Enteritidis. Among the food-borne pathogens, these bacteria accounted for the largest number of outbreaks, cases and deaths. According to the report of EFSA, there were 5,609 recorded food-borne outbreaks in Europe in 2007 alone. In these outbreaks, there were 3,291 hospitalization and 19 deaths. Primary cause of these cases was *Salmonella* spp. which was responsible for 1,773 hospitalization and 10 deaths (EFSA 2009). However, generally many cases of food-borne illness are not reported because patients do not seek medical care (Mead, et al. 1999).

Antimicrobial activities of Turkish EVOOs were tested against these three food-borne pathogenic bacteria. In order to eliminate the possibility of antimicrobial activity of fatty acids, refined olive, hazelnut and canola oils, which have similar fatty acid composition with virgin olive oils, were also investigated. Moreover, individual antimicrobial activities of ten phenolic compounds which exist in tested EVOO samples were examined. In order to investigate the combinational activities of phenolics, interactions between vanillin, vanillic acid, cinnamic acid and tyrosol were analyzed with two-level factorial design.

For complex molecules such as food, the measurement of antioxidants cannot be evaluated satisfactorily by a simple test; therefore, several methods may be required. For this reason, antioxidant activities of oil samples were evaluated by two different spectrophotometric methods, β-carotene-linoleic acid model system and ABTS radical scavenging method. Finally, results were correlated with total phenolic contents (TPC) of oil samples.

CHAPTER 3

MATERIALS AND METHODS

3.1. Oil Samples

Extra virgin olive oils from different parts of Aegean regions of Turkey were provided by Tariş. These oils belong to Dalaman, Koçarlı, Ödemiş, Gömeç, Altınoluk and Burhaniye. Different varieties of Turkish olive oils were produced from Erkence, Memecik and Nizip olives.

Erkence and Memecik olives are native to the west coast of Turkey. On the other hand, Nizip is a high oil producing cultivar from south-east of Turkey. The olives were hand-picked randomly at the same maturity level in 2006 harvest year (Ocakoglu 2008).

In the scope of the project of TUBITAK-TOVAG (No:104 O 333), olive oils were extracted from collected olive fruits by a 5 kg capacity laboratory scale olive mill (TEM Spremoliva, Italy) in the Department of Food Engineering at Izmir Institute of Technology. The phenolic compound profiles of each oil samples were determined by reversed phase HPLC/DAD analysis (Ocakoglu 2008).

Refined oil samples which are refined olive oil (Tariş, Izmir), hazelnut oil (Çotanak, Ordu) and canola oil (Olin, Edirne) were purchased from local markets. They were produced in 2008.

Oil samples in dark bottles were stored at 8°C and the headspaces were replaced by nitrogen after each use in order to prevent oils from deterioration. About 20 minutes prior to analyses, the bottles are placed into water bath at 20°C to warm them up to room temperature.

3.2. Bacterial Culture Preparation

Bacteria (*E. coli* O157:H7, *L. monocytogenes* and *S.* Enteritidis) were supplied from National Culture Type of Collection (NCTC, United Kingdom). Different medium was used for each bacterium: Lauria broth (LB) and LB agar (Agar, Merck) for *E. coli*, brain heart infusion broth (BHI, Fluka) and BHI agar (Fluka) for *L. monocytogenes* and tryptic soy broth (TSB, Fluka) and TSB agar (TSA, Merck) for *S.* Enteritidis. Lauria broth was prepared with yeast extract (Fluka), tryptone (Fluka) and sodium chloride (Riedel-deHaen). A single colony of bacteria was inoculated in appropriate medium. The overnight culture was transferred to fresh medium and incubated until the culture has reached to exponential phase. By the help of spectrophotometric measurement and viable cell count methods, the bacterial cultures were prepared in desired concentrations.

3.3. Analysis of Antimicrobial Activity

3.3.1. Antimicrobial Activity of Oils

The antimicrobial activity of oils was determined according to the method described by Medina et al. (2006) with minor modifications. Test tubes were prepared as in Table 3.1., and inoculated with a bacterial culture in concentrations of $5x10^4$ and $1x10^5$ cfu/ml. Therefore, the final concentrations of bacterial culture were $2.5x10^3$ and $5x10^3$ cfu/ml in the test tubes. In order to examine the antimicrobial effect of buffer extract of olive oil, test tubes containing olive oil and sterilized Phosphate-Buffered Saline Tween 20 (PBST) were shaken for one hour at 200 rpm at 37°C and centrifuged at 2000 rpm (Sigma, Germany) for 1 min. Then, the aqueous phase, free of oil was transferred into another test tube, and inoculated with the bacterial culture. All tubes were shaken in an orbital shaker for one hour at 200 rpm inside a 37°C incubator (GLF, Germany). After treatment, survivors were determined by viable cell count method.

According to previous analysis, EVOOs from Burhaniye and Nizip displayed the highest and the lowest TPC values, respectively. These two oils were tested with the same procedure except that the solutions were inoculated with a $2x10^6$ cfu/ml of bacteria and shaken for 30 minutes before the plating for viable cell count. The shake time of buffer extract of olive oil preparation was also 30 minutes. As a result, the antimicrobial activities of oils were calculated by comparing viable cell counts of treated samples with positive controls.

Finally, refined olive oil, hazelnut and canola oils were also tested with the same procedure with a $2x10^6$ cfu/ml of inoculum for 1 hour treatment time in order to compare antimicrobial activities of all oils.

Table 3.1. Contents of test tubes

SUBSTANCES	**TEST TUBES**		
	Control	**Oil –Buffer Extract**	**Oil**
PBST	1.9 ml	1 ml	0.95 ml
Oil	-	1 ml	1 ml
Bacterial Culture	100 µl	-	50 µl

3.3.2. Antimicrobial Activities of Phenolic Compounds

3.3.2.1. Phenolic Compound Solutions

Some of the phenolic compounds found in olive oil samples are vanillin (Fluka, PN:94750), vanillic acid (Fluka, PN:94770), cinnamic acid (Fluka, PN: 96340), tyrosol (Sigma-Aldrich, PN:188255), 4-hydroxybenzoic acid (Fluka, PN:54630), syringic acid (Fluka, PN:86230), luteolin (Sigma-Aldrich, PN:L9283), chlorogenic acid (Fluka, PN:25700), o-coumaric acid (Fluka, PN:28170), and ferulic acid (Fluka, PN:46278) which were tested for antimicrobial activities in this study. The solutions of each

compound were prepared in various concentration ranges given in Table 3.2. according to the HPLC analysis results (Appendix F). In addition, some of them were tested in higher concentrations since generally their levels in olives are higher than those in oils. Each of the compounds was firstly dissolved in ethanol, and then diluted with the appropriate medium to the target concentration. Ethanol content of all solutions was decreased below 1% (vol/vol) during dilutions. All solutions were prepared fresh before the experiments.

Table 3.2. Concentration ranges of phenolic compound solutions used in this study

Phenolic Compound	**Concentration (mg/kg oil)**									
4-Hydroxybenzoic acid	0.02	0.05	0.10	0.15	0.20	**2.00***	**20.0***	-	-	-
Tyrosol	1.0	2.5	4.0	5.5	7.0	8.5	**85***	**170***	**425***	**850***
Chlorogenic acid	0.08	0.16	**1.60***	**16.0***	**160***	-	-	-	-	-
Vanillic acid	0.05	0.10	0.15	0.20	0.25	**25.0***	-	-	-	-
Vanillin	0.05	0.10	0.20	0.35	0.50	**5.00***	**50.0***	**1386***	-	-
Syringic acid	0.10	0.20	0.30	0.40	0.80	**8.0***	-	-	-	-
Ferulic acid	0.10	0.25	0.40	0.55	**5.50***	**55.0***	-	-	-	-
O-coumaric acid	0.04	**0.40***	**4.00***	**40.0***	-	-	-	-	-	-
Cinnamic acid	0.05	0.10	0.50	1.00	1.50	2.00	-	-	-	-
Luteolin	0.5	1.0	1.5	2.0	2.5	3.0	-	-	-	-

*The bold values are higher concentrations than those found in EVOOs.

3.3.2.2. Determination of Individual Antimicrobial Activity of Phenolics

Antimicrobial activities of phenolic compounds were determined by microtitre plate method spectrophotometrically (Dufour, et al 2003). Dilutions were carried out in an appropriate broth medium. A 100 µl of each phenolic solution was dispensed into a well of flat bottom 96-well microtiter plate (Bio-Grainer, Germany). A 100 µl of $1x10^4$

cfu/ml bacterial cultures in logarithmic growth phase was added to each well. As a blank for each test solution, medium without bacteria, and phenolic solution in the same ratio (vol/vol) were filled into the wells. As a control, only bacterial culture and an appropriate medium was added. Then, absorbance measurements of each plate were taken in 3 hours intervals by Thermo Multiscan Spectra Reader (Finland) at 600 nm during incubation at 37°C for 24 hours.

3.3.2.3. Determination of Logarithmic Reduction of *L. monocyotogenes* Exposed to Cinnamic Acid

According to the percent inhibition results of phenolic compounds, logarithmic reduction of *L. monocytogenes* exposed to cinnamic acid at concentration of 2 mg/kg oil was examined. The microstate plate was prepared as in the method for antimicrobial activities of phenolic compounds. The growth of *L. monocytogenes* was observed by measuring the absorbance in 4 hours intervals for 24 hours. At the same time, the solutions in the wells were plated after the incubation time of 0, 8, 12, 16 and 24 hours. Bacterial enumeration in each sampling time point was determined by viable cell count method. Then, the log reduction was evaluated by comparing the obtained data with positive control.

3.3.2.4. Combinational Antimicrobial Activities

The method for individual antimicrobial activities of phenolic compounds was modified to determine the synergistic interactions between four phenolic compounds (k=4); tyrosol, vanillin, vanillic acid and cinnamic acid. Two-level factorial design was applied with 9 center points (CP=9) and 3 replications (n=3) of each treatments. Total of 57 experiments were run ($N = n2^k + CP = 3.2^4 + 9 = 57$). All combinations were presented in Table 3.3.

The solutions of phenolic compounds were prepared as explained in section 3.3.2.1 at the concentrations given in Table 3.3. A 25 µl of each solution and 100 µl of

bacterial culture were added to each well to obtain final volume of 200 µl as explained previously. All tests were performed with the appropriate controls similar to the method explained in determination of individual antimicrobial activities section. The absorbance measurements were taken at 600 nm in 3 hours intervals during incubation at 37°C for 24 hours.

Table 3.3. The phenolic compound concentrations used in the combination test

	Phenolic Compound Concentrations (mg/kg oil)			
Combination	**Vanillin**	**Vanillic Acid**	**Cinnamic Acid**	**Tyrosol**
1	1.0	0.1	0.5	1
2	1.0	0.1	3	1
3	1.0	0.1	0.5	10
4	1.0	0.1	3	10
5	1.0	1	0.5	1
6	1.0	1	3	1
7	1.0	1	0.5	10
8	1.0	1	3	10
9	0.1	0.1	0.5	1
10	0.1	0.1	3	1
11	0.1	0.1	0.5	10
12	0.1	0.1	3	10
13	0.1	1	0.5	1
14	0.1	1	3	1
15	0.1	1	0.5	10
16	0.1	1	3	10
Center Point	0.55	0.55	1.75	5.5

3.4. Analysis of Antioxidant Activity

3.4.1. Methanolic Extraction of Olive oil

In order to prepare methanolic extracts of oils, 2 g of each oil sample, 10 ml of 80% v/v methanol and 30 µl of Tween 20 (Sigma-Aldrich) were homogenized at 15000

rpm (Heidolph Silent Crusher M Homogenizer, Germany) for 1 min. The obtained mixture was centrifugated at 5000 rpm for 10 min at room temperature. Supernatant was collected in a graduated cylinder and the oil phase in tube was transferred to the beaker again. The homogenization and centrifugation processes were repeated twice with the same oil sample. At the end, about 30 ml of methanolic extract collected in the graduated cylinder. Extracts were freshly prepared before each experiment.

3.4.2. β-Carotene Bleaching Assay

The antioxidant activity of olive oil extracts was evaluated by the β-carotene linoleate model system (Deba, et al. 2008, Medina, et al. 2006). β-carotene (2.0 mg) (Sigma, PN:C9750) was dissolved in 10 ml chloroform. 1 ml of the β-carotene solution was mixed with 20 µl linoleic acid (Sigma, PN:L1376) and 200 mg Tween 40 (Sigma, PN:P1504). The chloroform was removed by evaporation under vacuum at 45°C, then 50 ml distilled water was added, and the mixture vigorously shaken to form a stable emulsion. The emulsions were freshly prepared before each experiment. An aliquot (250 µl) of β-carotene-linoleic acid emulsion and methanolic extracts (30 µl) of olive oils were dispensed into each of the 96-wells of microtitre plates. Methanolic solution of butylated hydroxyanisol (BHA, 25-50 ppm) (Sigma-Adrich) and methanol were also used as a standard and the control, respectively. The plates were incubated at 45°C, and their absorbance were measured at 460 nm against a blank consisting of methanol and the emulsion without β-carotene by Thermo Multiscan Spectra Reader (Finland). As soon as the samples were added to the wells, the zero time absorbance was measured and measurements were taken for every 15 min until 180 min during incubation at 45°C. Experiment was performed three times with three replicates for each sample.

The antioxidant activity (AA) of oil extracts was evaluated in terms of bleaching of the β-carotene using the following formula,

$$AA = 100\,[1-(A_0-A_t) / (A'_0-A'_t)] \qquad (3.1)$$

where A_0 and A'_0 are the absorbance values at zero time of the incubation for sample and control, respectively, and A_t and A'_t are the absorbance values measured for sample and control, respectively, after incubation for 180 min.

3.4.3. ABTS Radical Scavenging Method

The determination of antioxidant activity by ABTS radical scavenging method was performed according to the procedure described by others (Sanchez, et al. 2007, Ling, et al. 2009). ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution (1.8 mM) (Sigma, PN:A3219) with 0.63 mM potassium persulfate (Fluka, PN:60490) (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Then, the solution was diluted with ethanol until absorbance reached to 0.700 (±0.030) at 734 nm. Measurements were performed at ambient temperature. Oil extracts were diluted at a ratio of 1:10 with methanol (80%). Later, 190 µl of radical solution was mixed with 10 µl of diluted extracts in a microtitre plate. The absorbance at 734 nm was measured for every 1 min until 13 min following initial mixing. Appropriate solvent blanks were run in each assay. BHA (2.5 ppm) and methanol (80%) were used as the standard antioxidant and the negative control, respectively. Experiments were performed three times with three replicates for each sample. The percent free radical scavenging activity was calculated according to the following formula;

$$\% \text{ Free Radical Scavenging Activity} = [(A_n - A_s) \times 100] / A_n \qquad (3.2)$$

where A_n is the final absorbance values of negative control, and A_s is the final absorbance values of sample.

3.5. Determination of Total Phenolic Contents of Refined Olive Oil, Hazelnut and Canola Oils

Total phenolic contents of refined olive oil, hazelnut and canola oils were determined by Folin-Ciocalteu method which is based on the reaction of a phosphowolframate-phosphomolybdate complex by phenolics to blue reaction products (Luther, et al. 2007). In a test tube, 1 ml of methanolic oil extract was diluted with 5 ml pure water. Then, 0.5 ml of Folin-Ciocalteu reagent (Fluka) and 2 ml of sodium carbonate solution (Reidel-deHaen) (15% w/v) were added. Mixture was diluted with water to 10 ml. After 2 h of reaction in dark at ambient temperature, absorbance was measured at 765 nm. The same protocol was repeated with 1 ml methanol (Sigma-Aldrich) (80%) for blank. In order to construct a calibration curve, standard solution of gallic acid (Fluka, PN:48630) (0.005 – 1 mg/ml) was used.

3.6. Statistical Analysis

Antimicrobial activity results of oil samples were calculated from the reduction in the numbers of bacteria which were transformed into $\log_{10}$. Standard deviation values of bacterial growth curves in the presence of phenolic compounds were not indicated in graphs, because values were between 0.002 and 0.31. Such low values were not separable since growth lines were too close to each other. The experimental data of combinational antimicrobial activity test were analyzed in a model with MODDE 8 (Umetrics, Umea, Sweden).

CHAPTER 4

RESULTS AND DISCUSSION

In the first part of this study, antimicrobial activities of Turkish EVOO samples, refined olive, hazelnut and canola oils were investigated against three food-borne pathogenic bacteria, *E. coli* O157:H7, *L. monocytogenes* and *S.* Enteritidis. The individual and combinational antimicrobial activities of phenolic compounds determined in EVOOs were assessed by microtiter plate assay. In the second part, the antioxidant activities of EVOO and refined oil samples were examined by two different assays which are β-carotene linoleic acid model system and ABTS radical scavenging method.

4.1. Antimicrobial Activity

4.1.1. Antimicrobial Activities of EVOOs, Refined Olive Oil, Hazelnut and Canola Oils

Antimicrobial properties of nine different types of EVOO samples and refined olive oil, hazelnut and canola oils were tested against three food-borne pathogens; *E. coli* O157:H7*, L. monocytogenes* and *S.* Enteritidis. Firstly, EVOO samples were tested with an inoculum at concentrations of either $5x10^4$ or $1x10^5$ cfu/ml with 1 h treatment time. As a result, there were no survivors after treatment. According to Folin-Ciocalteu analysis, Burhaniye and Nizip EVOOs have showed the highest and the lowest TPC values, respectively (Appendix F). Therefore, these EVOOs were treated with higher concentration of culture ($2x10^6$ cfu/ml) for 1 h period of time. In addition, refined oils were tested with same conditions. While EVOOs showed bactericidal activity, refined olive, hazelnut and canola oils did not cause any significant decrease in microbial

population. This difference could be because of the fact that virgin olive oils contain high concentration of phenolic compounds but refined oils do not. Furthermore, treatment time was decreased to 30 min and tests were repeated with Burhaniye and Nizip EVOOs, and they decreased the number of bacteria by 5 logs even in such a shorter time. The all results were summarized in Table 4.1.

Table 4.1. Antimicrobial activity results of EVOOs, refined olive oil, hazelnut and canola oils for different inoculum concentrations and treatment times

Oil Sample	Inoculum Concentration	Treatment Time	Result
EVOOs	$1x10^5 – 5x10^4$ cfu/ml	1 h	**NO Survivors**
Burhaniye	$2x10^6$ cfu/ml	1 h	**NO Survivors**
Nizip	$2x10^6$ cfu/ml	1 h	**NO Survivors**
Refined Olive Oil	$2x10^6$ cfu/ml	1 h	0.00-0.30 log reduction
Hazelnut Oil	$2x10^6$ cfu/ml	1 h	0.10-0.37 log reduction
Canola Oil	$2x10^6$ cfu/ml	1 h	0.10-0.18 log reduction
Burhaniye	$2x10^6$ cfu/ml	30 min	**NO Survivors**
Nizip	$2x10^6$ cfu/ml	30 min	**NO Survivors**

In general, olive fruit contains higher amount of phenolic compounds then its oil. For instance, it has been reported that total phenolic content of Tunisian table olives were between 3390 and 18010 mg GA/kg (Othman, et al. 2008). In another study, antimicrobial activity of table olives has been investigated and the phenolic contents of the samples were from 900 to 5000 mg/kg. As a result, samples inhibited the growth of tested organisms which were *Bacillus cereus, Bacillus subtilis, Staphylococus aureus, E. coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Candida albicans and Cryptoccus neoformans* (Pereira, et al. 2006). Previously, the range of total phenolic content of the EVOO samples used in our study had been found between 125 and 353 mg GA/kg oil (Ocakoglu 2008). This shows that EVOOs having such a low TPC are able to inhibit *E. coli* O157:H7, *L. monocytogenes* and *S.* Enteritidis. Similar to our

findings, Medina et al. (2006) reported that Spanish virgin olive oils had strong bactericidal activity against *L. monocytogenes* and *Salmonella enterica*; however, they displayed weaker activity against *E. coli*. Moreover, other edible vegetable oils (sunflower, corn, rapeseed, cotton and soybean) had no effect against all tested organisms.

4.1.2. Individual Activity of Phenolic Compounds

Activities of ten phenolic compounds, vanillic, ferulic, o-coumaric, chlorogenic, cinnamic, 4-hydroxybenzoic, and syringic acids, and tyrosol, luteolin, vanillin were analyzed against *E. coli* O157:H7*, L. monocytogenes* and *S.* Enteritidis by microtiter plate assay. According to results of absorbance measurements during 24 h incubation, the growth curves in the presence of each phenolic compound were plotted as O.D. (Optical Density) versus time (hour). The growth curves of *E. coli* O157:H7, *L. monocytogenes* and *S.* Enteritidis in the presence of several concentrations of each phenolic compound were given in Appendix A., Appendix B., and Appendix C, respectively. The most distinctive results were given as example in Figure 4.1., Figure 4.2. and Figure 4.3, which are the growth curves of *E. coli* O157:H7, *L. monocytogenes* and *S.* Enteritidis in the presence of syringic acid, ferulic acid and luteolin, respectively. The standard deviation values of these graphs are lower than 0.01, 0.01 and 0.008, respectively. All experiments were carried out in duplicates.

The activity levels of different concentrations of compounds can be distinguished significantly from the control in the growth curves after 9 and 15 hours of incubation. The slope of the growth curves for *E. coli* and *L. monocytogenes* changed after 9 hour of incubation, and the lines separated from each other. For *Salmonella*, similar changes occurred after 12 hours of incubation. The antimicrobial activities of compounds were determined ideally from the O.D. values at time point of 24 h.

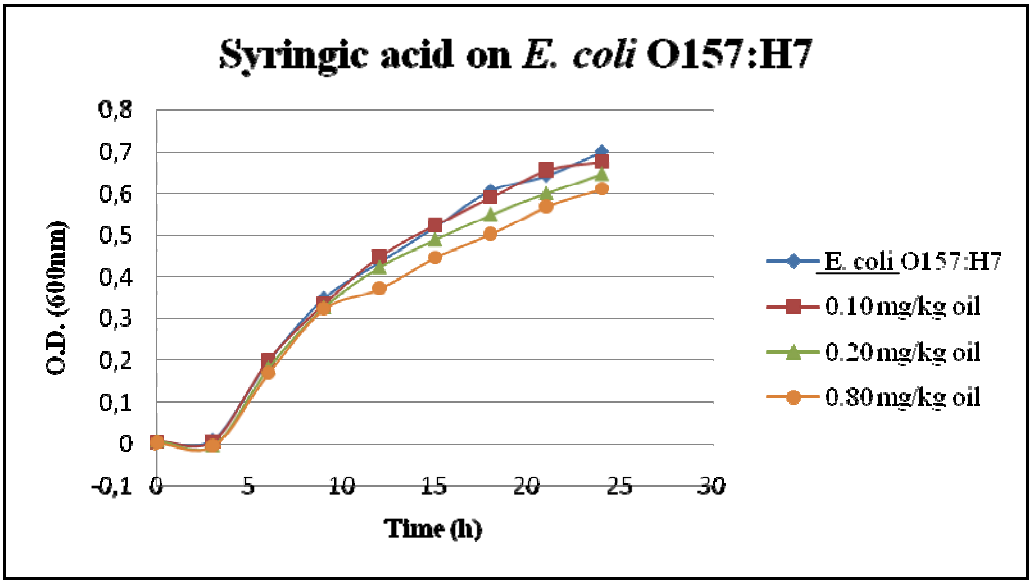

Figure 4.1. The growth of *E. coli* O157:H7 in the presence of syringic acid

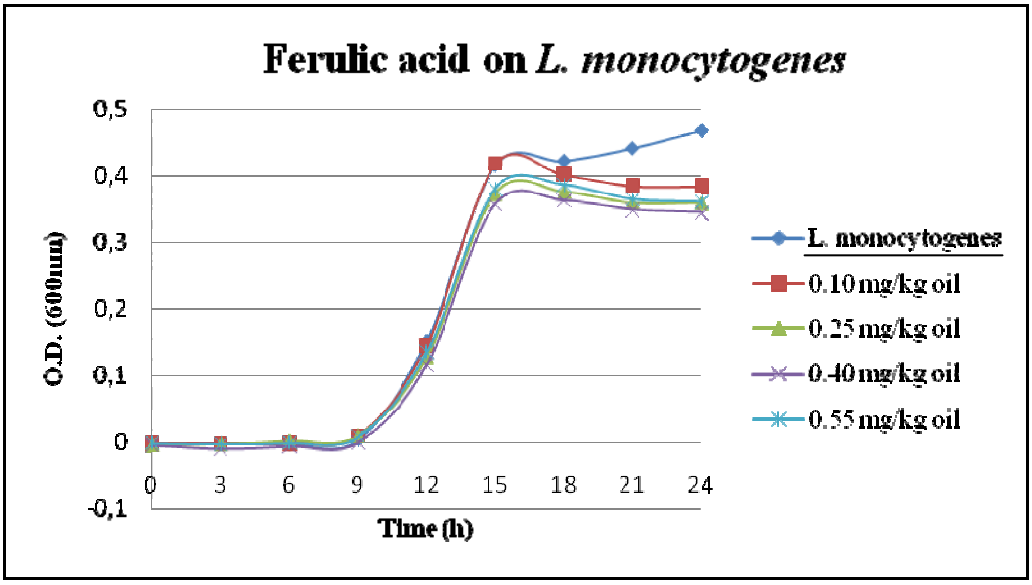

Figure 4.2. The growth of *L. monocytogenes* in the presence of ferulic acid

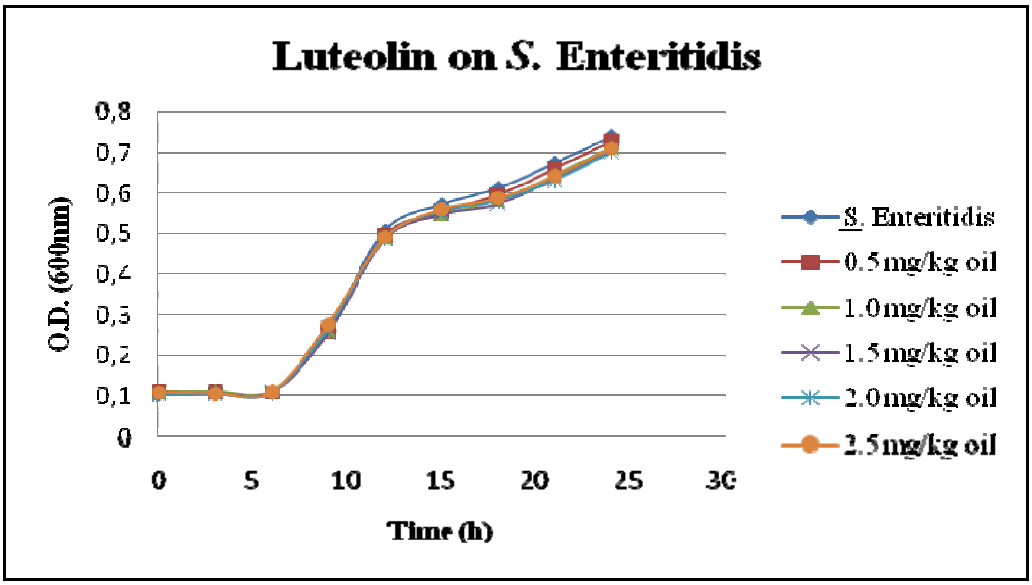

Figure 4.3. The growth of *S.* Enteritidis in the presence of luteoline

The percent inhibitions in growth rate at the end of 24 hour incubation period were calculated for each phenolic compound against *E. coli* O157:H7 (Table 4.2.), *L. monocytogenes* (Table 4.3.) and *S.* Enteritidis (Table 4.4). These values were calculated by the reduction in optical cell density of each sample with reference to the positive control.

Among all the data for *E. coli* O157:H7, vanillin at concentration of 1286 mg/kg oil gave the highest percent inhibition (80%) in the growth rate (Table 4.2.). However, this concentration is much higher than that found in an extra virgin olive oil. The second highest activity was observed in the presence of tyrosol at concentration of 850 mg/kg oil. However, this concentration is about 100 times higher than the highest concentration (8.6 mg/kg oil, Koçarlı) determined in tested EVOO samples. On the other hand, the highest percent inhibition (27.68%) in the growth of *L. monocytogenes* was observed when cinnamic acid was used at the concentration of 2.00 mg/kg oil which is in the range of what was found in the EVOOs (Table 4.3.).

Furthermore, it was found that the effect of ferulic acid in the concentration ranges determined in EVOOs was not significant against *E. coli* O157:H7 (Table 4.2.) and *S.* Enteritidis (Table 4.4.). However, it showed higher effect against *L. monocytogenes* (Table 4.3.). In addition, higher concentrations (5.5 and 55.0 mg/kg oil) of ferulic acid gave rise to only 3% of inhibition in growth of *E. coli* and *S.* Enteritidis,

while they caused 12-16% of inhibition on *L. monocytogenes.* Almost all tested phenolic compounds showed higher activity against *L. monocytogenes,* but a weaker effect against *E. coli* O157:H7 and *S.* Enteritidis (Table 4.5.). It seemed that phenolic compounds were more active against Gram positives than Gram negatives. It has been reported that Gram positives are more prone to the action of oil extracts than Gram negatives and our finding supports this (Medina, et al. 2006).

The reported minimum concentrations of phenolic compounds that inhibit bacterial growth are much higher than those found in olive oils. The antimicrobial activities of ferulic, vanillic, p-coumaric and 4-hydroxybenzoic acids which are also found in wine were tested on *C. jejuni* with the treatment time of 15 min, and then survivors determined by viable cell count. 4-hydroxybenzoic acid was found very effective at the lowest concentration (1 mg/L). While vanillic acid was effective at the concentration as low as 10 mg/L, ferulic and p-coumaric acid could show activity at the concentration of 100 mg/L which is not normally present in wines. An important point, in this study, is that the all tested solutions contained 5% ethanol, and this could be the reason for such high activity (Ganan, et al. 2009).

Table 4.2. The percent inhibition in the growth rate of *E. coli* O157:H7 in the presence of phenolic compounds

Phenolic Compound	Concentration mg/kg oil	% Inhibition
Cinnamic acid	0.05	2.05
	0.10	4.62
	1.00	5.39
	1.50	6.00
Clorogenic acid	0.08	4.72
	1.60	5.12
	16.0	8.39
	160	12.17
O-coumaric acid	0.04	2.40
	0.40	4.70
	4.00	6.69
	40.0	11.07
Ferulic acid	5.5	3.90
	55	3.97
Luteolin	1.0	2.64
	1.5	2.86
	2.0	4.38
Syringic acid	0.1	3.74
	0.2	7.81
	0.8	9.88
	8.0	12.80

Phenolic Compound	Concentration mg/kg oil	% Inhibition
Tyrosol	1.0	1.02
	2.5	1.09
	4.0	2.82
	5.5	4.30
	7.0	5.79
	8.5	6.29
	425	7.65
	850	17.61
Vanillic acid	0.05	6.93
	0.15	11.50
	0.20	13.13
Vanillin	0.05	7.49
	0.10	7.51
	0.20	8.39
	0.35	10.42
	0.50	10.52
	1286	80.16
4-hydroxybenzoic acid	0.02	2.04
	0.05	4.95
	0.10	6.44
	0.15	8.47
	20.0	10.33

Table 4.3. The percent inhibition in the growth rate of *L. monocytogenes* in the presence of phenolic compounds

Phenolic Compound	Concentration mg/kg oil	% Inhibition
Cinnamic acid	0.05	6.93
	0.10	14.29
	0.50	17.34
	1.00	19.36
	1.50	23.23
	2.00	27.68
Clorogenic acid	0.08	3.71
	1.60	5.16
	16.0	11.21
	160	19.42
O-coumaric acid	0.04	8.97
	0.40	9.67
	4.00	22.78
	40.0	23.85
Ferulic acid	0.10	2.12
	0.25	8.14
	0.40	9.68
	0.55	9.99
	5.5	12.53
	55	16.06
Luteolin	0.5	5.29
	1.0	7.51
	1.5	10.90
	2.0	11.49
	2.5	15.45
	3.0	19.05

Phenolic Compound	Concentration mg/kg oil	% Inhibition
Syringic acid	0.1	9.14
	0.2	9.92
	0.3	12.12
	0.4	13.38
	0.8	15.28
	8.0	21.09
Tyrosol	1.0	3.54
	2.5	12.27
	5.5	12.63
	7.0	13.16
	8.5	13.45
	85	19.55
	170	20.50
Vanillic acid	0.05	6.97
	0.10	9.39
	0.15	13.78
	0.20	14.22
	0.25	15.96
	25.0	23.20
Vanillin	0.05	7.63
	0.10	11.15
	0.20	11.37
	0.35	12.03
	0.50	14.88
	5.00	18.22
4-hydroxybenzoic acid	0.10	19.53
	0.15	20.69

Table 4.4. The percent inhibition in the growth rate of *S.* Enteritidis in the presence of phenolic compounds

Phenolic Compound	Concentration mg/kg oil	% Inhibition
Cinnamic acid	0.05	8.27
	0.10	9.70
	0.50	11.14
	1.50	11.02
	2.00	11.13
Clorogenic acid	0.08	1.57
	0.16	2.52
	1.60	4.05
	16.0	5.33
	160	9.09
O-coumaric acid	0.04	4.85
	0.40	9.80
	4.00	11.29
Ferulic acid	0.25	0.96
	0.55	0.97
	5.50	3.25
	55.0	3.71
Luteolin	0.5	1.73
	1.0	3.84
	1.5	4.52
	2.0	5.70

Phenolic Compound	Concentration mg/kg oil	% Inhibition
Syringic acid	0.1	3.24
	0.2	5.65
	0.3	5.72
	0.4	6.80
	0.8	7.94
Tyrosol	1.0	5.27
	2.5	10.47
	4.0	10.52
	5.5	11.38
	7.0	11.64
	8.5	11.20
Vanillic acid	0.05	0.25
	0.10	2.67
	0.15	6.86
	0.20	6.80
Vanillin	0.05	0.75
	0.10	3.10
	0.20	3.24
	0.35	4.83
4-hydroxybenzoic acid	0.05	4.57
	0.10	6.80
	0.15	10.86

Table 4.5. Comparison of percent inhibition values of o-coumaric acid, chlorogenic acid, vanillin and tyrosol against *E. coli* O157:H7, *L. monocytogenes* and *S.* Enteritidis. The bold values indicate the highest percent inhibitions for each phenolic.

		% Inhibition in Growth Rate		
Phenolic Compound	Concentration mg/kg oil	*E. coli* O157:H7	*Listeria monocytogenes*	*Salmonella* Enteritidis
O-coumaric acid	0.04	2.40	8.97	4.85
	0.40	4.70	9.67	9.80
	40.0	11.07	**23.85**	11.29
Clorogenic acid	0.08	4.72	3.71	1.57
	1.60	5.12	5.16	4.05
	16.0	8.39	11.21	5.33
	160	12.17	**19.42**	9.09
Vanillin	0.05	7.49	7.63	0.75
	0.10	7.51	11.15	3.10
	0.20	8.39	11.37	3.24
	0.35	10.42	**12.03**	4.83
Tyrosol	1.0	1.02	3.54	5.27
	2.5	1.09	12.27	10.47
	5.5	4.30	12.63	11.38
	7.0	5.79	13.16	11.64
	8.5	6.29	**13.45**	11.20

In the report of Rodriguez Vaquero et al. (2007) one of the analyzed phenolic compounds was vanillic acid which was tested against *L. monocytogenes.* An inhibition of 10%, 13%, 22% and 37% in the final cell density after 18 h of incubation was observed by the addition of vanillic acid in concentrations of 50, 100, 200 and 500 mg/L, respectively, in the presence of 5% ethanol. In our study, vanillic acid at a concentration of 25 mg/kg oil (~23 mg/L, with ethanol content lower than 0.1% (vol/vol)) was tested against *L. monocytogenes*. As a result, 4.95% and 23% inhibition was observed after 18 h and 24 h incubation times, respectively. Our results are in agreement with this previous report.

In the report of Tunçel and Nergis (1993), minimum inhibitory concentrations of o-coumaric, ferulic, vanillic, syringic acids and tyrosol against *E. coli* O157:H7 were

determined as 450, 450, 550, 550 and 600 μg/ml respectively by agar dilution method. These concentrations are about 1000 times higher from the levels of those in EVOOs.

In our study, while all EVOO samples showed bactericidal activity against all three food-borne pathogens, the tested phenolic compounds have slight antimicrobial activity. The reason for this result could be the tested phenolic compound concentrations which were too low to show stronger activity by themselves. Moreover, it seems that since olive oil contains more than 30 different phenolic compounds in its composition, an increase of their overall effect can be due to the synergistic interactions or/and due to the sum of their individual antimicrobial effects are also very possible.

Furthermore, our results indicate that the activities of all tested compounds were concentration dependent. The findings of the antimicrobial activities of the phenolic compounds were comparatively represented in the Figure 4.4, Figure 4.5, and Figure 4.6 for *E. coli* O157:H7, *L. monocytogenes* and *S.* Enteritidis, respectively. Almost all results showed that the activities are concentration dependent but not directly proportional. For example, tyrosol in concentration of 7 mg/kg showed 5.79% inhibition in the growth of *E. coli*, while the concentration of 850 mg/kg which is 120 times of 7 mg/kg, did only 17.61% inhibition (Table 4.2.).

Although the concentration of vanillin was increased from 0.35 to 0.50 mg/kg oil, antimicrobial activity against *E. coli* O175:H7 didn't show any significant change. The findings were also similar for ferulic acid against this organism. While its concentration increased 10 times (from 5.5 to 55 mg/kg oil), the activity was increased only from 3.90% to 3.97% (Table 4.2.).

The antimicrobial activity of 0.04 mg/kg oil of o-coumaric acid decreased the growth of *L. monocytogenes* by 8.97%. When concentration increased by 1000-fold (40.0 mg/kg oil), the antimicrobial activity didn't change proportionally, and increased only about 3-fold (Table 4.3.).

The most effective phenolic compounds for *S.* Enteritidis were cinnamic acid (from 0.50 to 2.00 mg/kg oil), o-coumaric acid (4.0 mg/kg oil) and tyrosol (5.5 and 7.0 mg/kg oil), which caused about 11% inhibition (Table 4.4.). Although the tested concentrations of cinnamic acid and tyrosol are among those concentration ranges found in EVOOs, the tested concentration of o-coumaric acid is 100 times higher than what found in EVOOs.

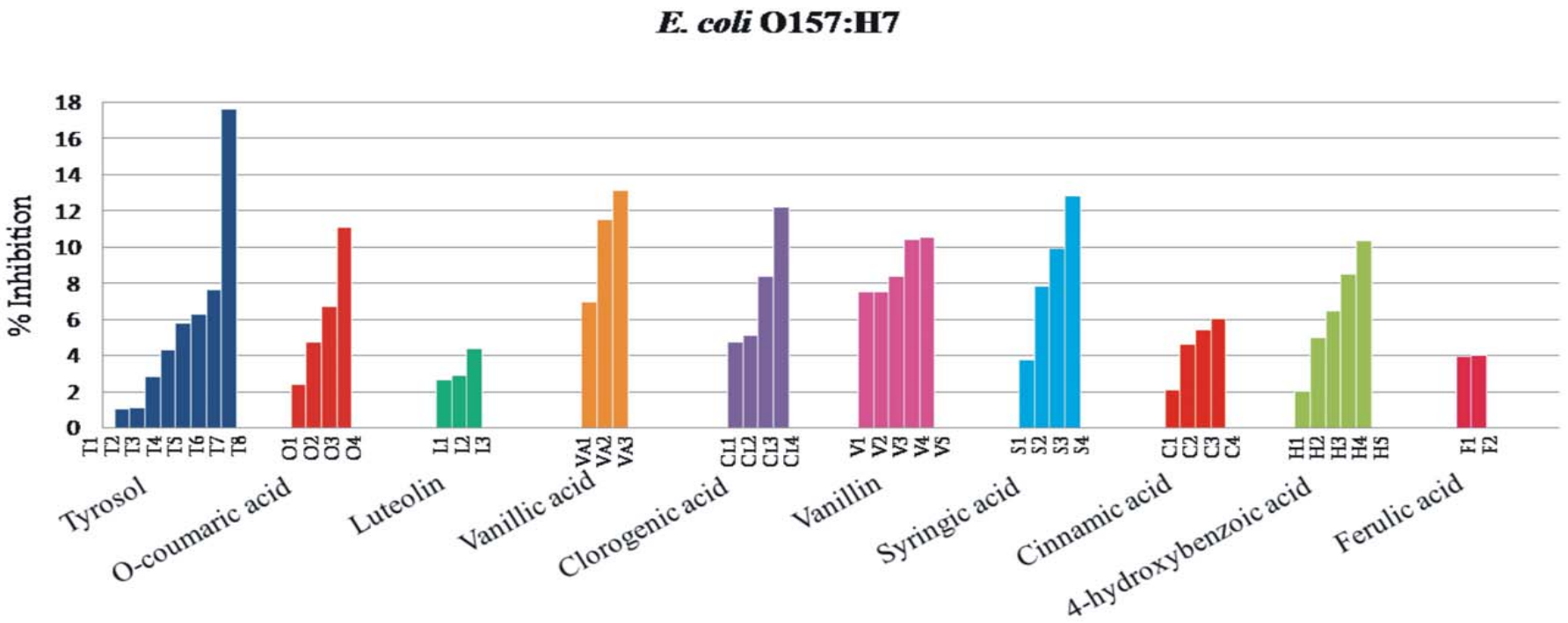

Figure 4.4. Concentration dependent activities of phenolic compounds against *E. coli* O157:H7. Tyrosol: T1, T2, T3, T4, T5, T6, T7, T8 correspond to 1.0, 2.5, 4.0, 5.5, 7.0, 8.5, 425, 850 mg/kg oil, O-coumaric acid: O1, O2, O3, O4 correspond to 0.04, 0.40, 4.00, 40.0 mg/kg oil, Luteolin: L1, L2, L3 correspond to 1.0, 1.5, 2.0 mg/kg oil, Vanillic acid: VA1, VA2, VA3 correspond to 0.05, 0.15, 0.20 mg/kg oil, Chlorogenic acid: Cl1, Cl2, Cl3, Cl4 correspond to 0.08, 1.60, 16.0, 160 mg/kg oil, Vanillin: V1, V2, V3, V4, V5 correspond to 0.05, 0.10, 0.20, 0.35, 0.50, Syringic acid: S1, S2, S3, S4 correspond to 0.1, 0.2, 0.8, 8.0 mg/kg oil, Cinnamic acid: C1, C2, C3, C4 correspond to 0.05, 0.10, 1.00, 1.50 mg/kg oil, 4-hydroxybenzoic acid: H1, H2, H3, H4, H5 correspond to 0.02, 0.05, 0.10, 0.15, 2.00 mg/kg oil, and Ferulic acid: F1, F2 correspond to 5.5, 55 mg/kg oil, respectively.

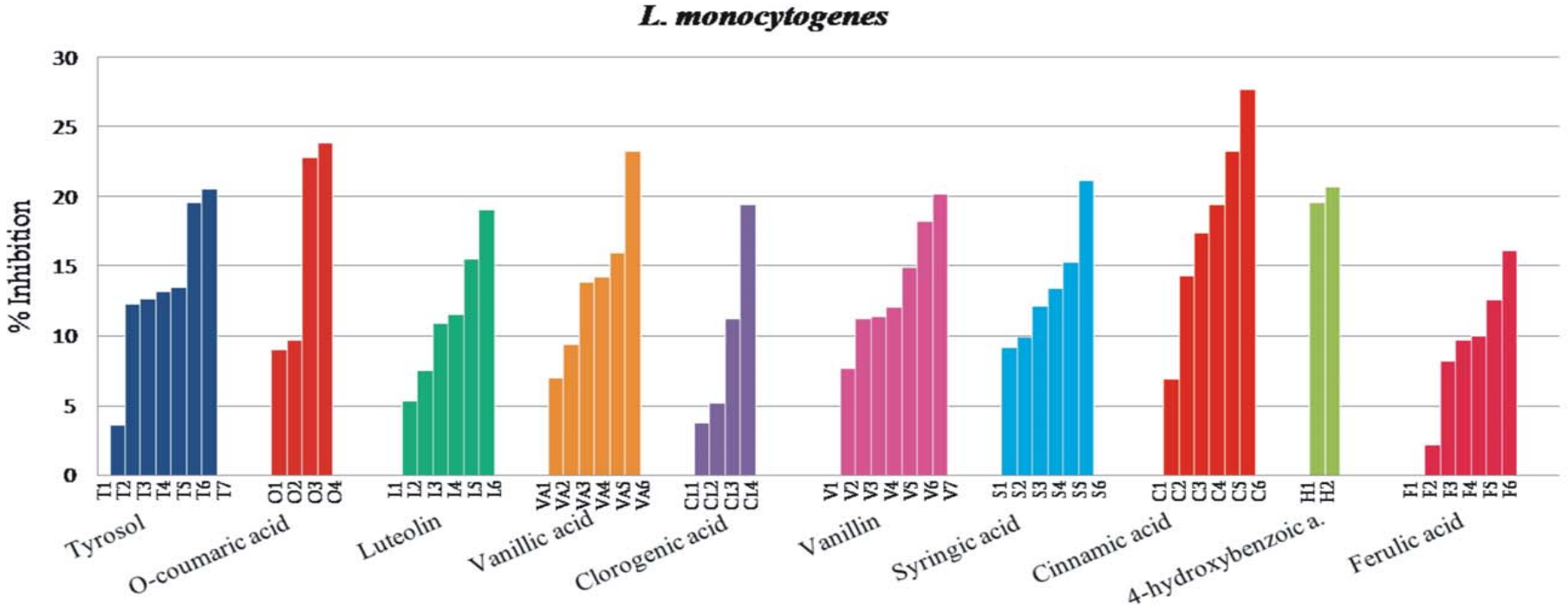

Figure 4.5. Concentration dependent activities of phenolic compounds against *L. mococytogenes*. Tyrosol: T1, T2, T3, T4, T5, T6, T7 correspond to 1.0, 2.5, 5.5, 7.0, 8.5, 85, 850 mg/kg oil, O-coumaric acid: O1, O2, O3, O4 correspond to 0.04, 0.40, 4.00, 40.0 mg/kg oil, Luteolin: L1, L2, L3, L4, L5, L6 correspond to 0.5,1.0, 1.5, 2.0, 2.5, 3.0 mg/kg oil, Vanillic acid: VA1, VA2, VA3,VA4,VA5, VA6 correspond to 0.05, 0.10, 0.15, 0.20, 0.25, 25.0 mg/kg oil, Chlorogenic acid: Cl1, Cl2, Cl3, Cl4 correspond to 0.08, 1.60, 16.0, 160 mg/kg oil, Vanillin: V1, V2, V3, V4, V5, V6, V7 correspond to 0.05, 0.10, 0.20, 0.35, 0.50, 5.00, 50.0, Syringic acid: S1, S2, S3, S4, S5, S6 correspond to 0.1, 0.2, 0.3, 0.4 0.8, 8.0 mg/kg oil, Cinnamic acid: C1, C2, C3, C4, C5, C6 correspond to 0.05, 0.10, 0.50, 1.00, 1.50, 2.00 mg/kg oil, 4-hydroxybenzoic acid: H1, H2 correspond to 0.10, 0.15 mg/kg oil, and Ferulic acid: F1, F2, F3, F4, F5, F6 correspond to 0.10, 0.25, 0.40, 0.55, 5.5, 55 mg/kg oil, respectively.

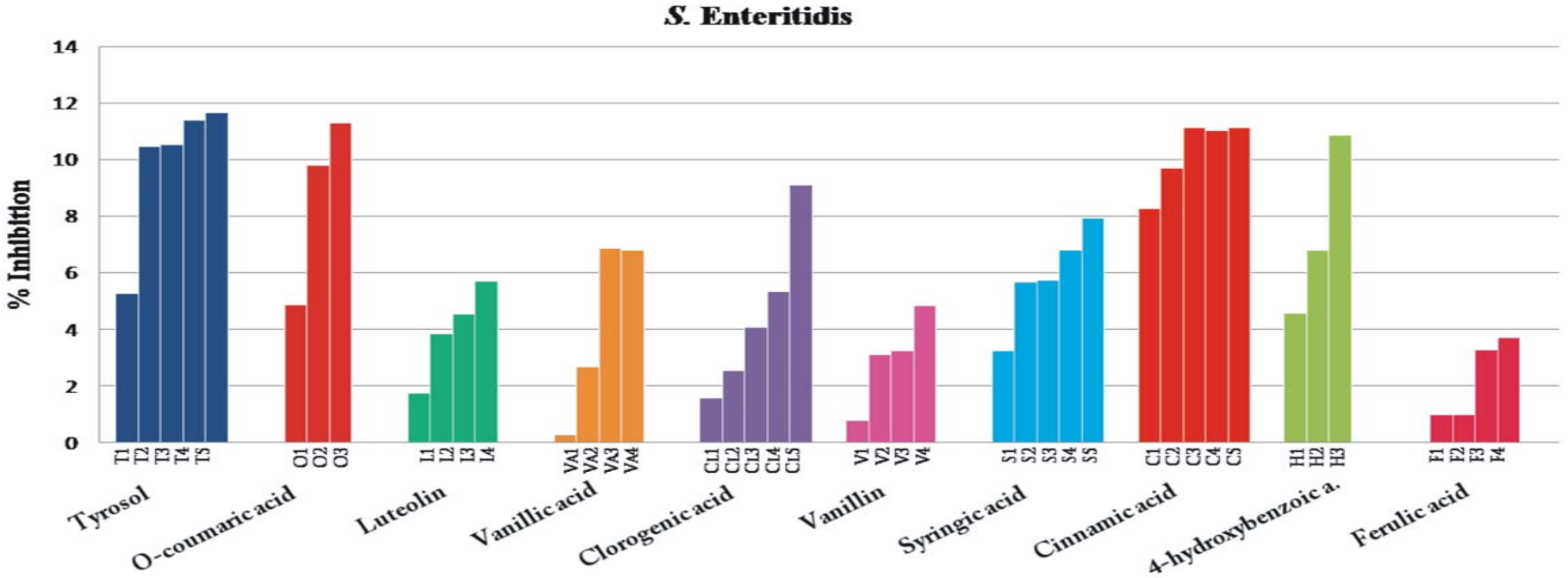

Figure 4.6. Concentration dependent activities of phenolic compounds against *S.* Enteritidis. Tyrosol: T1, T2, T3, T4, T5 correspond to 1.0, 2.5, 4.0, 5.5, 7.0 mg/kg oil, O-coumaric acid: O1, O2, O3 correspond to 0.04, 0.40, 4.00 mg/kg oil, Luteolin: L1, L2, L3, L4 correspond to 0.5, 1.0, 1.5, 2.0 mg/kg oil, Vanillic acid: VA1, VA2, VA3, VA4 correspond to 0.05, 0.10, 0.15, 0.20 mg/kg oil, Chlorogenic acid: Cl1, Cl2, Cl3, Cl4, Cl5 correspond to 0.08, 0.16, 1.60, 16.0, 160 mg/kg oil, Vanillin: V1, V2, V3, V4 correspond to 0.05, 0.10, 0.20, 0.35 mg/kg oil, Syringic acid: S1, S2, S3, S4, S5 correspond to 0.1, 0.2, 0.3, 0.4, 0.8 mg/kg oil, Cinnamic acid: C1, C2, C3, C4, C5 correspond to 0.05, 0.10, 0.50, 1.50, 2.00 mg/kg oil, 4-hydroxybenzoic acid: H1, H2, H3 correspond to 0.05, 0.10, 0.15 mg/kg oil, and Ferulic acid: F1, F2, F3, F4 correspond to 0.25, 0.55, 5.5, 55 mg/kg oil, respectively.

By nature most phenolic compounds are soluble only in organic solvents which are the primary limitation for antimicrobial studies. However, some like vanillin can be dissolved in water, which makes it possible to experiment higher concentrations in such studies. In Figure 4.7., the antimicrobial effect of vanillin in various concentrations, including the 1386 mg/kg oil, against *E. coli* is given. Rupasinghe et al. (2006) reported that the minimum inhibitory concentration of vanillin against *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter aerogenes* (ATCC 13048), *Salmonella enterica* subsp. *enterica* serovar Newport (ATCC 6962), *Candida albicans* (ATCC 10231), *S. cerevisiae* (ATCC 9763), *Penicillum expansum* (ATCC 7861), and *Lactobacillus casei* (ATCC 7469) were between 6 mM (1040 mg/kg oil) and 12 mM (2079 mg/kg). Such high concentrations can be tested because vanillin can be dissolved in water in contrast to other compounds. In agreement with our results (Figure 4.7.), Rupasinghe et al. (2006) demonstrated that 6 mM (1040 mg/kg oil) vanillin caused to 73% inhibition in the growth rate of *E. coli.* In this study, 8 mM (~1386 mg/kg oil) vanillin caused 80% inhibition in the growth rate of *E. coli* O157:H7. In addition, such high concentration of vanillin showed a bacteriositatic effect against *E. coli* O157:H7 during 15 h of incubation.

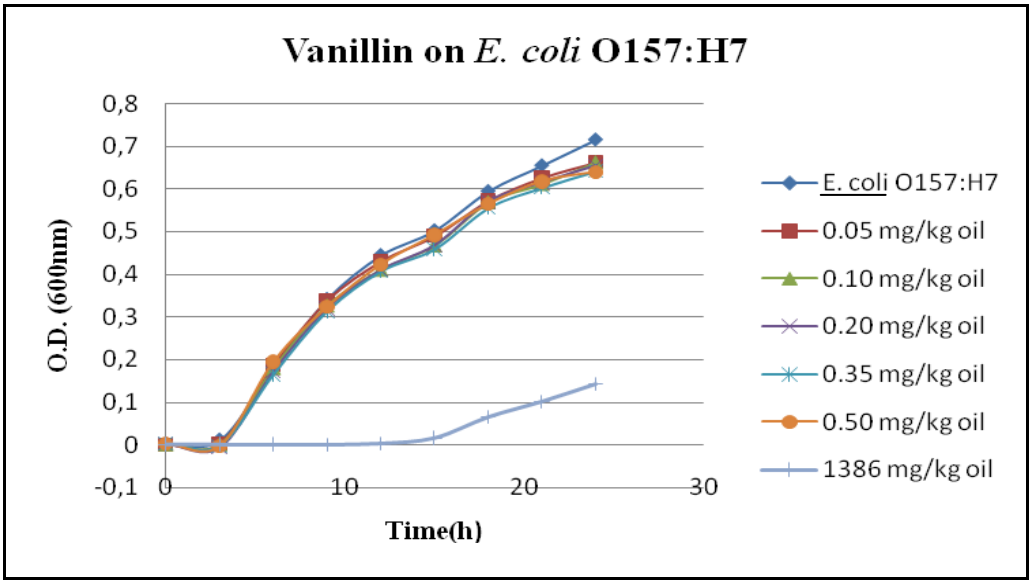

Figure 4.7. The growth of *E. coli* O157:H7 in the presence of vanillin in concentrations of 0.05, 0.10, 0.20, 0.35, 0.50 and 1386 mg/kg oil.

4.1.3. Logarithmic Reduction of *L. monocyotogenes* Exposed to Cinnamic Acid

The level of cinnamic acid in Erkence EVOO was determined as 1.98 mg/kg oil which was the highest cinnamic acid concentration among all EVOO samples. Also, among all tested phenolic compounds, cinnamic acid (2.00 mg/kg oil) showed the highest individual antimicrobial activity against *L. monocytogenes* with 27% inhibition in the growth rate (Table 4.3.). Therefore, the level of reduction in the number of bacteria was investigated during 24 hours of incubation. The logarithmic reduction values are given in Table 4.6.

Table 4.6. Log reduction in the number of *L. monocytogenes* in the presence of 2 mg/kg oil cinnamic acid at the time points of 0, 8, 12, 16 and 24 h

Time (h)	Log Reduction
0	0.18
8	0.07
12	0.10
16	0.14
24	0.25

Although cinnamic acid in concentration of 2.00 mg/kg oil showed the highest percent inhibition (27%), it could decrease the number of bacteria by 0.25 logs at the end of 24 hours incubation. In comparison with cinnamic acid, it can be concluded that the other phenolic compounds have very low log reduction effect.

4.1.4. Combinational Activity of Tyrosol, Vanillin, Vanillic and Cinnamic Acids

Two-level factorial design was applied to reveal the main and interaction effects between vanillin (V), vanillic acid (VA), cinnamic acid (Cin) and tyrosol (Tyr) (k=4) with 9 center point (CP=9) and 3 replications (n=3) of each treatments. Experimental

data were given in Appendix D. The ANOVA table and list of coefficients with p-values are given in Table 4.7.

Factors and interactions with $p<0.05$ were considered significant. The main factors that had significant interactions were kept in the model due to model hierarchy.

The interactions between vanillic acid-cinnamic acid, vanillic acid-tyrosol and cinnamic acid-tyrosol were found significant. According to the results the high inhibition were found at the combinations where one phenolic compound at low level while the second were at the high level. Moreover, only vanillin was found significant as individual effect.

Table 4.7. The ANOVA table and list of coefficients with p-values

Factors and Interactions	P-value
Constant	0
V	0.025
VA	0.172
Cin	0.45
Tyr	0.411
VA*Cin	0
VA*Tyr	0
Cin*Tyr	0

ANOVA

% Inhibition	DF	SS	MS (variance)	F	p
Total	56	2415.96	43.1422		
Constant	1	1559.48	1559.48		
Total Corrected	55	856.486	15.5725		
Regression	7	528.997	75.571	11.0764	0
Residual	48	327.489	6.82269		
Lack of Fit	9	34.8217	3.86908	0.515583	0.854
(Model Error)					
Pure Error	39	292.667	7.50429		
(Replicate Error)					

N = 56	Q2 = 0.502	Cond. no. =	1.08
DF = 48	R2 = 0.618	Y-miss =	0
	R2 Adj. = 0.562	RSD =	2.612
		Conf. lev. =	0.95

4.2. Total Phenolic Contents of Refined Olive Oil, Hazelnut and Canola Oils

The total phenolic contents of EVOOs were previously determined in another study (Ocakoglu 2008). In this study, the total phenolic contents of refined oils, which are refined olive oil, hazelnut and canola oils, were analyzed. As expected, their phenolic content was found lower than EVOOs, because refining process causes loss of phenolic compounds (Gunstone 2002). In a study on determination of total phenolic contents of EVOO, olive oil and highly refined olive oil, it has been reported that TPC values for EVOO samples were between 73 and 265 mg GA/kg oil, and were between 14 and 30 mg GA/kg oil for olive oil and TPC of refined olive oil was 4 mg GA/kg oil (Pellegrini, et al. 2001). The standard curve and TPC values were given in Appendix E. and Table 4.8., respectively.

Table 4.8. Total phenolic content (TPC) values of refined olive, hazelnut and canola oils

Oil Sample	TPC (mg GA/kg oil)
Refined Olive Oil	91.67 ± 1.30
Hazelnut Oil	41.67 ± 5.18
Canola Oil	58.88 ± 2.68

4.3. Antioxidant Activities of Oils

4.3.1. Antioxidant Activity by β-Carotene Bleaching Assay

Olive oil contains numerous compounds having antioxidant activity such as tocopherols, β-carotene, and especially phenolic compounds. In many studies, it was demonstrated that there is a correlation between total phenol content and antioxidant activity of oils (Gorinstein, et al. 2003, Sanchez, et al. 2007).

In β-carotene-linoleic acid method, the antioxidant activity level of a substance is determined by measuring oxidation products of linoleic acid which simultaneously attack β-carotene, resulting in bleaching of its characteristic yellow color (Gorinstein, et al. 2003, Fukumoto and Mazza 2000).

Table 4.9 lists the results of β-carotene-linoleic acid method for various oil samples. Activities of EVOO samples varied between 21.22% and 64.54%. These results are consistent with previous reports (Gorinstein, et al. 2003, Sanchez, et al. 2007). In these studies, antioxidant activities were also found about 40% for EVOOs. In this study, Erkence EVOO, which has the highest TPC, showed the highest antioxidant activity. Refined olive, hazelnut and canola oils have lower antioxidant activity values and this might be attributed to low TPC content of these oils. Although Nizip EVOO has higher phenolic content than refined oils, its antioxidant activity is among the level of refined oils. This might be due to different contents of other antioxidant molecules such as tocopherols. It has been reported that canola oils have considerably high tocopherol contents despite the refining process (Gunstone 2002).

Table 4.9. The antioxidant activity (%AA) results of EVOOs, refined olive oil, hazelnut and canola oils, and standard solution (BHA). (Standard deviation values are between 0.65 and 9.91.)

Oil Sample	**%AA**
Erkence	64.54
Burhaniye	60.58
Koçarlı	57.93
Ödemiş	42.91
Dalaman	38.49
Gömeç	45.54
Altınoluk	53.38
Memecik	29.83
Nizip	21.22
Refined olive	23.50
Canola	22.52
Hazelnut	21.19
Standard	
BHA 50 ppm	85.43
BHA 25ppm	74.76

The correlation of the total phenolic content and the antioxidant activity was not very high (R^2=0.8075). This might be due to the sensitivity of methods. It is previously reported that β-carotene method was the worst method which gave low correlation with TPC (R^2=0.7258) (Sanchez, et al. 2007). In contrast, another study showed that a high correlation was achieved for β-carotene method (R^2=0.9958) (Gorinstein, et al. 2003).

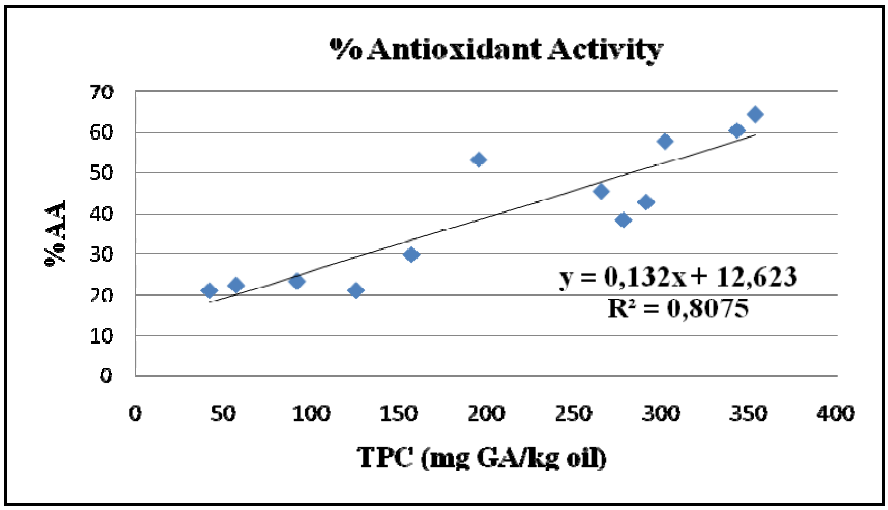

Figure 4.8. Correlation between antioxidant activity results of β-carotene method and total phenol contents (TPC) of oil samples

4.3.2. Free Radical Scavenging Activity by ABTS Method

The $ABTS^{\bullet+}$ scavenging capacity method is a decolorization assay that measures the capacity of antioxidants directly reacting with $ABTS^{\bullet+}$ radicals generated by a chemical method (Yu 2008). The percent free radical scavenging activity (FRSA) values were calculated by the help of absorbance values (Table 4.10.). FRSA values were varied between 1.31% and 21.97%. As in the β-carotene method, refined oils have lower activity by ABTS method. Moreover, results have higher correlation with TPC values, and R^2 of 0.9082 was obtained compared to β-carotene method (Figure 4.9.). This result is consistent with a previous report which was a study of determining of the antioxidant activity of olive oil by four different methods; ABTS, DPPH, ORAC and β-

carotene methods (Sanchez, et al. 2007). In their report, it was reported that the best correlation was given by ABTS method (R^2=0.8927) and the worst was given by the β-carotene method (R^2=0.7258)

Table 4.10. The percent free radical scavenging activity (%FRSA) results of EVOOs, refined olive oil, hazelnut and canola oils, and standard solution (BHA). Standard deviation values are between 0.20 and 3.34

Oil Sample	%FRSA
Erkence	21.97
Burhaniye	16.68
Koçarlı	13.95
Ödemiş	13.20
Dalaman	11.79
Gömeç	10.70
Altınoluk	9.93
Memecik	5.34
Nizip	5.60
Refined olive	3.95
Canola	1.55
Hazelnut	1.31
Standard	
BHA 2.5 ppm	6.35

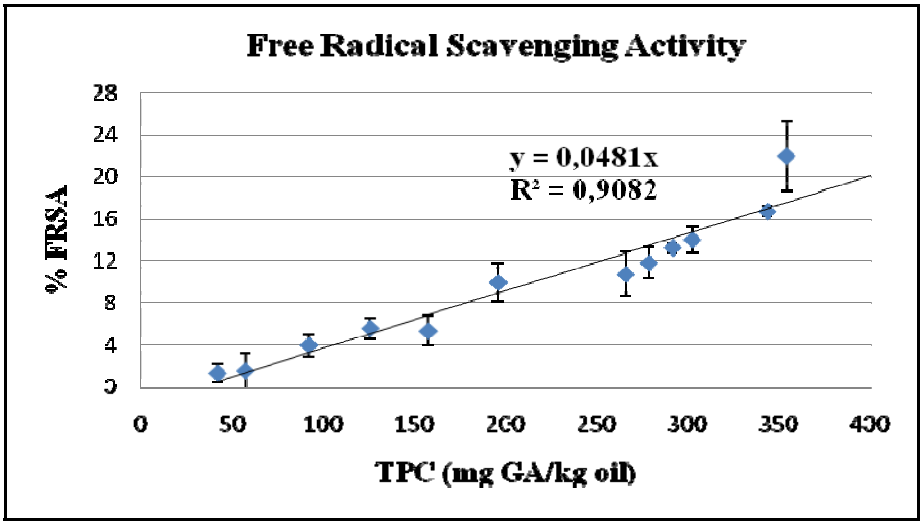

Figure 4.9. Correlation between free radical scavenging activity results of ABTS method and total phenol contents (TPC) of oil samples

CHAPTER 5

CONCLUSION

The aim of this study is to investigate the antimicrobial and antioxidant properties of different varieties of Turkish extra virgin olive oils compared to refined olive oil, canola and hazelnut oils. An important point is that there is no published research about antimicrobial and antioxidant properties of Turkish olive oils. As far as we know, this is the first study on antimicrobial and antioxidant properties of different varieties of Turkish EVOOs with respect to refined olive, hazelnut and canola oils.

In antimicrobial activity part, activities of oils and their phenolic compounds were tested against *E. coli* O157:H7, *L. monocytogenes* and *S*. Enteritidis. As a result, while EVOOs showed strong bactericidal activity, refined oils were found as ineffective. Moreover, phenolic compounds showed very low antimicrobial activity individually. Their activities are calculated as percent inhibition in growth rate according to that of control which is the growth of bacteria without phenolic compound. The percent inhibition results of the phenolic compounds in EVOO ranges between 1.03% and 13.13% for *E. coli* O157:H7, 2.12% and 27.68% for *L. monocytogenes*, and 0.25% and 11.64% for *S.* Enteritidis. Among these three food-borne bacteria, *L. monocytogenes* was the most sensitive against phenolic compounds. For example, cinnamic acid at a concentration of 1.50 mg/kg oil showed 23.23% inhibition against *L. monocytogenes*, while that against *S.* Enteritidis and *E. coli* O157:H7 remained at 11.02% and 6.00%, respectively. In addition, concentration of 2.00 mg/kg oil showed the highest percent inhibition for *L. monocytogenes*. When degree of logarithmic reduction was investigated for this concentration, a reduction in the number of bacteria by only 0.25 logs was observed. This shows that phenolic compounds have very slight antimicrobial activity at these low concentrations although EVOOs showed strong antimicrobial activity. However, it is possible that there might be an increase of their overall effect due to the synergistic interaction or/and due to the sum of their individual antimicrobial effects.

In order to examine the synergistic interactions of phenolic compounds, tyrosol, vanillin, vanillic and cinnamic acids were tested by two-level factorial design. While the interactions between vanillic acid-cinnamic acid, vanillic acid-tyrosol and cinnamic acid-tyrosol were found significant, only vanillin was found significant as individual effect. Moreover, the high microbial inhibition was obtained at the combinations where one phenolic compound at low level while the second was at the high level. Since olive oil contains more than 30 different phenolic compounds in its composition, there could be more synergistic interactions.

For determination of antioxidant activities of oil samples, β-carotene - linoleum acid model system and ABTS method were performed and results were compared with a standard antioxidant (BHA). It was found that antioxidant activity values are varied between 21.19% and 64.54% by β-carotene method. According to ABTS method, free radical scavenging activity values varied between 1.31% and 21.97%. Furthermore, EVOOs showed higher antioxidant activities while refined oils have lower activity as expected since phenolic compounds present in higher concentration in EVOOs than those in refined oils. Results of both β-carotene and ABTS methods showed correlation with TPC values of oils, R^2=0.8075 and 0.9082, respectively.

REFERENCES

Anetta, P., B. W. Baumstark, P. Marckmann, H. Gylling, B. Sandstrom. 2002. An olive oil-rich diet results in higher concentrations of LDL cholesterol and a higher number of LDL subfraction particles than rapeseed oil and sunflower oil diets. *Journal of Lipid Research* 41: 1901-1911.

Attaran, A., N. MacDonald, M.B. Stanbrook, B. Sibbald, B.J.K. Flegel, R. Kale, P.C. Hebert. 2008. Listeriosis is the least of it. *CMAJ* 179:739-740.

Banerjee, Ruma. 2008. *Redox Biochemistry*. New Jersey: Wiley – Interscience Press.

Bhunia, Arun K. 2008. *Foodborne Microbial Pathogens: Mechanisms and Pathogenesis.* New York: Springer Science Press.

Bisignano, G., A. Tomaino, R. L. Cascio, G. Crisafi, N. Uccella, A. Saija. 1999. On the In-vitro Antimicrobial Activity of Oleuropein and Hydroxytyrosol. *Journal of Pharmacy and Pharmacology* 51:971-974.

Benedetto, R. D., V. Rosaria, S. Beatrice, F. Carmela, S. Carmela, G. Claudio, M. Paola, D. A. Massimo, M. Roberta 2007. Tyrosol, the major extra virgin olive oil compound, restored intracellular antioxidant defences in spite of its weak antioxidative effectiveness. *Nutrition, metabolism, and cardiovascular diseases* 17:535-545.

Black, Jacquelyn G. 2004. *Microbiology- principles and explorations.* New York: Wiley Press.

Blekas, G., E. Psomiadou, M. Tsimidou, D. Boskou. 2002. On the importance of total polar phenols to monitor the stability of Greek virgin olive oil. *European Journal of Lipid Science and Technology* 104:340-346.

Boskou, Dimitrios. 2006. *Olive Oil: Chemistry and Technology*. Illinois: AOCS Press.

Boskou, Dimitrios. 2009. *Olive oil- Minor Constituents and Health*. New York: CRC Press.

Brenes, M., F. Hidalgo, A. Garcia, J. Rios, P. Garcia, R. Zamora, A. Garrido. 2000. Pinoresinol and 1-acetoxypinoresinol, two new phenolic compounds identified in olive oil. *Journal of the American Oil Chemists' Society* 77:715-720.

Cao, G., E. Sofic, R.L. Prior. 1997. Antioxidant and Prooxidant Behavior of Flavonoids: Structure-Activity Relationships. *Free Radical Biology and Medicine* 22:749-760.

Centers for Disease Control and Prevention. 2008. (CDC 2008-a) http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5714a2.htm (accessed 2009)

Centers for Disease Control and Prevention. 2008. (CDC 2008-b) http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salment_g.htm (accessed 2009)

Coia, J.E. 1998. Clinical, microbiological and epidemiological aspects *of Escherichia coli* O157 infection. *FEMS Immunology and Medical Microbiology* 20:1-9.

Deba, F., T. D. Xuan, M. Yasuda, S. Tawata. 2008. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from Bidens pilosa Linn. var. Radiata. *Food Chemistry* 19:346–352.

Di Tommaso, D., R. Calabrese, D. Rotilio. 1998. Identification and Quantitation of Hydroxytyrosol in Italian Wines. *Journal of High Resolution Chromatography* 21:549-553.

Doyle, Michael P., Larry. R. Beuchat, Thomas J. Montville. 2001. *Food microbiology: Fundamentals and Frontiers*. Washington: ASM Press.

Duffy, G., E. Cummins, P. Nally, S. O'Brein, F. Butler. 2006. A review of quantitative microbial risk assessment in the management of *Escherichia coli* O157:H7 on beef. *Meat Science* 74:76-88.

Dufour, M., R. S. Simmonds, P. J. Bremer. 2003. Development of a method to quantify in vitro the synergistic activity of "natural" antimicrobials. *International Journal of Food Microbiology* 85:249-258.

European Food Safety Autorithy. 2009. (EFSA 2009). The Community Summary Report on Food-borne Outbreaks in the European Union in 2007. *The EFSA Journal* 271.

Fabiani, R., A. De Bartolomeo, P.Rosignoli, M. Servili, G.F. Montedoro, G. Morozzi. 2002. Cancer chemoprevention by hydroxytyrosol isolated from virgin olive oil through G1 cell cycle arrest and apoptosis. *European journal of cancer prevention* 11:351-358.

Finotti, E. and D. Di Majo. 2003. Influence of solvents on the antioxidant property of flavonoids. *Nahrung/Food* 47:186-187.

Fito, M. R. De la Torre, M. Farre-Albaladejo, O. Khymenetz, J. Marrugat, M. I. Covas. 2007. Bioavailability and antioxidant effects of olive oil phenolic compounds in humans: a review. *Ann Ist Super Sanità* 43:375-81.

Food and Drug Administration. 2007. (FDA 2007-a) Listeria monocytogenes. In Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. http://www.cfsan.fda.gov/~mow/chap6.html (accessed 2009).

Food and Drug Administration. 2007. (FDA 2007-b) Salmonella Spp. In Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5714a2.htm (accessed 2009).

Ganan, M., A. J. Martinez-Rodriguez, A.V. Carrascosa 2009. Antimicrobial activity of phenolic compounds of wine against *Campylobacter jejuni. Food Control* 20:739-742.

Gandhi, M and M. L. Chikindas. 2007. Listeria: A foodborne pathogen that knows how to survive. *International Journal of Food Microbiology* 113:1-15.

Glass, K. A., J.M. Loeffelhoslz, J. P. Ford, M. P. Doyle. 1992. Fate of Escherichia-Coli O157/H7 as Affected by Ph or Sodium-Chloride and in Fermented, Dry Sausage. *Applied and Environmental Microbiology* 58:2513-2516.

Gorinstein, S., O. Martin-Belloso, E. Katrich, A. Lojek, M. Ciz, N. Gligelmo-Miguel, R. Haruenkit, Y.S. Park, S.T. Junk, S. TRackhtenberg. 2003. Comparison of the contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils as determined by four different radical scavenging tests. *The Journal of Nutritional Biochemistry* 14:154-159.

Göğüş, Fahrettin, Mucahit T. Ozkaya, and Semih Otleş, eds. 2009. *Zeytinyağı.* Ankara: Eflatun Yayınevi.

Gunstone, Frank. D. eds. 2002. *Vegetable Oils in Food Technology: Composition, Properties and Uses*. Boca Raton, Fla: CCR Press.

Haddada, F. M., D. Krichene, H. Manai, I. Oueslati, D. Daoud, M. Zarrouk. 2008. Analytical evaluation of six monovarietal virgin olive oils from Northern Tunisia. *European Journal of Lipid Science and Technology* 110:905-913.

Heyndrickx, M., F. Pasmans, R. Ducatela, A. Decostere, F. Haesebrouk. 2005. Recent changes in *Salmonella* nomenclature: The need for clarification. *The Veterinart Journal* 170:275-277.

International Olive Oil Council. 2008. (IOOC 2008) Designations and definitions of olive oils. http://www.internationaloliveoil.org/web/aa-ingles/oliveWorld/aceite1.html (accessed 2008)

Jaen. 2005. International conference on the healthy effect of virgin olive oil. *European Journal of Clinical Investigation* 35:421-424.

Karch, H., P.I. Tarr, M. Bielaszewska. 2005. Enterohaemorrhagic *Escherichia coli* in human medicine. *International Journal of Medical Microbiology* 295:405-418.

Kiosseoglou, V. and P. Kouzounas. 1993. The role of diglycerides, monoglycerides and free fatty acids in olive oil minor surface-active lipid interaction with proteins at oil-water interfaces. *Journal of Dispersion Science and Technology* 14:527 - 539.

Ling, L. T., S. A. Yap, A. K. Radhakrishnan, T. Subramaniam. 2009. Standardized *Magifera indica* extract is an ideal antioxidant. *Food Chemistry* 113: 1154-1159.

Lui, D., M.L. Lawrence, L. Gorski, R.E. Manrell, A. J. Answorth, F. W. Austin. 2006. *Listeria monocytogenes* serotype 4b strains belonging to lineages I and III possess distinct molecular features. *J. Clin. Microbiol.* 44:214-217.

Luther, M., J. Parry, J. Moore, J. Meng, Y. Zhang, Z. Cheng, L. Lucy yu. 2007. Inhibitory effect of Chardonny and black raspberry seed extracts on lipid oxidation in fish oil and their redical scavenging and antimicrobial properties. *Food Chemistry* 104:1065-1073

Low, J. C. and W. Donachie. 1997. A review of *Listeria monocytogenes* and listeriosis. *The Veterinary Journal* 153:9-29.

Madigan, Michael T., John Martinko, and Jack Parker, eds. 2002. *Brock Biology of Microorganisms*. Ney Jersey: Prentice Hall.

Manna, C., P. Galletti, V. Cucciolla, G. Montedoro, V. Zappia. 1999. Olive oil hydroxytyrosol protects human erythrocytes against oxidative damages. *The Journal of Nutritional Biochemistry* 10:159-165.

Masella, R., R. Vari, M. DArchivio, R. Di Benedetto, P. Matarrese, W. Malorni, B. Scazzochio, C. Giovannini. 2004. Extra Virgin Olive Oil Biophenols Inhibit Cell-Mediated Oxidation of LDL by Increasing the mRNA Transcription of Glutathione-Related Enzymes. *J. Nutr*. 134:785-791.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg Infect Dis* 5:607-25.

Medina, E., E. De Castro, C. Romero, M. Brenes. 2006. Comparison of the concentrations of phenolic compounds in olive oils and other plant oils: correlation with antimicrobial activity. *J. Agric. Food Chem.* 54:4954-4961.

Moghadasian, M. H. 2000. Pharmacological properties of plant sterols *in vivo* and *in vitro* observations. *Life Sciences* 67:605-615

Nightingale, K. K., K. Windham, M. Weidmann. 2005. Evolution and molecular phylogeny of *Listeria monocytogenes* isolated from human and animal Listeriosis cases and foods. *J. Bacteriol.* 187:5537-5551.

Ocakoglu, D. 2008. Classification of Turkish virgin olive oils based on their phenolic profiles. *Izmir Institute of Technology, Food Engineering Programme* 47-93

Othman, N. B., D. Roblain, P. Thonart, M. Hamdi. 2008. Tunusian table olive phenolic compounds and their antioxidant capacity. *Food Chemistry* 73:235-240

Pamplona, R., G. Barja, M. Porterotin. Membrane fatty acid unsaturation, protection against oxidative stress, and maximum life span. *Annals of the New York Academy of Sciences* 959:475-490.

Panza, F., V.Solfrizz, A. M. Colacicco, A. D'Introno, C. Capurso, F. Torres, A. Del Parigi, S. Capurda, A. Capursa. 2004. Mediterranean diet and cognitive decline. *Public Health Nutrition* 7:959-963.

Pellegrini, N., F. Visioli, S. Buratti, F. Brighenti. 2001. Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. *Journal of Agricultural and Food Chemistry* 49:2532-2538.

Pereira, J. A.,A.P.G. Pereira, I.G.F.R. Ferreira, P.Valentap, P.B. Andrade, R. Seabra, L. Estevinho, A. Bento. 2006. Table olives from Portugal; phenolic compounds, antioxidant potential and antimicrobial activity. *Journal of Agricultural and Food Chemistry* 54:8425-8431.

Perez-Camino, M. C., W. Moreda, A. Cert. 2001. Effects of olive fruit quality and oil storage practices on the diacylglycerol content of virgin olive oils. *Journal of Agricultural and Food Chemistry* 49:699-704.

Radford, S. A., R. G. Board, 1995. The influence of sodium chloride and pH on the growth of *Salmonella enteritidis* PT4. *Lett. Appl. Microbiol.* 20:11-3.

Ramaswamy, V., V. M. Crescence, J. S. Rejitha, M. U. Lekhsmi, K. S. Dharsana, S. P. Prasad, H. M. Vijila. 2007. Listeria-Review of epidemiology and pathogenesis. *Journal of Microbial Immunology Infection* 40:4-13.

Ray, Bibek eds. 2003. *Fundamental Food Microbiology*: New York: CRC Press.

Rice-Evans, C. A., N. J. Miller, G. Paganga. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine* 20:933-956.

Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Heber, E. S. Johnson, N. T. Hargett, P. A. Blake, M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* 308:681-685.

Rodriguez Vaquero, M. J., M.R. Alberto, M. C. Manca de Nadra. 2007. Influence of phenolic compounds from wines on the growth of *Listeria monocytogenes*. *Food control* 18:587-593.

Romero, C., E. Medina, J. Vargas, M. Brenes, A. De Castro. 2007. In vitro activity of olive oil polyphenols against *Helicobacter pylori*. *Journal of Agricultural and Food Chemistry* 55:680-686.

Rupasinhe, H. P. V., J. Boulter-Bitzer, T. Ahn, J. A. Odumeru. 2006. Vanillin inhibits pathogenic and spoilage microorganisms in vitro and aerobic microbial growth in fresh-cut apples. *Food Research International* 39:575-580.

Ryan, D., M. Antolovich, P. Prenzler, K. Robards, S. Lavee. 2002. Biotransformations of phenolic compounds in Olea europaea L. *Scientia Horticulturae* 92:147-176.

Ryser, Eliot T., and Elmer H. Marth, eds 2007. *Listeria, Listeriosis and Food Safety.* New York: CRC Press.

Salami, M., C. Galli, L. De Angelis, F. Visioli. 1995. Formation of F2-isoprostanes in oxidized low density lipoprotein: Inhibitory effect of hydroxytyrosol. *Pharmacological Research* 31:275-279.

Sanchez, C. S., A. M. T. Gonzalez, M. C. Garcia-Parrilla, J. J. Q. Granados, H. L. G. de la Serrana, M. C. L. Martinez. 2007. Different radical scavenging tests in virgin olive oil and their relation to the total phenol content. *Analytica Chimica Acta* 593:103–107.

Schikora, A., A. Carrei, E. Charpentier, H. Hirt. 2008. The dark side of the salads: *Salmonella typhimurium* overcomes the innate immune response of *Arabidopsis thaliana* and shows an endopathogenic lifestyle. *Plos One* 3: 2279

Simopoulos, Artemis P., and Francesco Visioli eds. 2007. *More on Mediterranean Diets*. Basel: Karger Press.

Soni, M. G., G. A. Burdock, M. S. Christian, C. M. Bitler, R. Crea. 2006. Safety assessment of aqueous olive pulp extract as an antioxidant or antimicrobial agent in foods. *Food and Chemical Toxicology* 44:903-915.

Spyros, A., A. Philippidis, P, Dais. 2004. Kinetics of diglyceride formation and isomerization in virgin olive oils by employing 31P NMR Spectroscopy. formulation of a quantitative measure to assess olive oil storage history. *Journal of Agricultural and Food Chemistry* 52:157-164.

Tilden, J. Jr., W.Young, A.M. MaNamara, C. Custer, B. Boesel, M. A. Lambert-Fair,J. Majkowski, D. Vugia, S. B. Werner, J. Hollingsworh, J. G. Morris, Jr. 1996. A new route of transmission for Escherichia coli: infection from dry fermented salami. *Am. J. Public Health* 86:1142-1145.

Trichopoulou, A. and V. Dilis. 2007. Olive oil and longevity. *Mol. Nutr. Food Res.* 51:1275-1278.

Tripoli, E., M. Giammanco, G. Tabacchi, D. Di Majo, S. Giammanco, M. La Guardia. 2005. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutrition Research Reviews* 18:98-112.

Tuck, L. K. and P. J. Hayball. 2002. Major phenolic compounds in olive oil: metabolism and health effects. *The Journal of Nutritional Biochemistry*. 13:636-644.

Tunçel. G. and C. Nergiz. 1993. Antimicrobial effect of some olive phenols in a laboratory medium. *Letters in Applied Microbiology* 17:300-302.

Vecchia, C. La. 2004. Mediterranean diet and cancer. *Public Health Nutrition* 7:965-968.

Vermerris, Wilfred and Ralph Nicholson eds. 2006. *Phenolic Compound Biochemistry*. Dordrecht: Springer Press.

Vierira, O., J. Laranjinha, V. Madeira, L. Almeida. 1998. Cholesteryl ester hydroperoxide formation in myoglobin-catalyzed low density lipoprotein oxidation: concerted antioxidant activity of caffeic and p-coumaric acids with ascorbate. *Biochemical Pharmacology* 55:333-340.

Visioli, F., A. Poli, C. Galli. 2002. Antioxidant and other biological activities of phenols from olives and olive oil. *Medicinal Research Reviews* 22:65-75.

Visioli, F., and C. Galli. 1998. The effect of minor constituents of olive oil on cardiovascular disease: new findings. *Nutrition Reviews* 56:142-147.

Visioli, F., D. Caruso, S. Grande, R. Bosisio, M. Villa, G. Galli, C. Sirtori, C. Galli. 2005. Virgin Olive Oil Study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. *European Journal of Nutrition* 44:121-127.

Waterman, E. and B. Lockwood. 2007. Active components and clinical applications of olive oil. *Altern. Med. Rev.* 12:331-42.

Weinbrenner, T., M. Fito, R. de la Torre, G. T. Saez, P. Rijken, C. Tormos, S. Coolen, M. F. Albaladejo, S. Abanades, H. Schroder, J. Marrugat, M. I. Covas. 2004. Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. *J. Nutr.* 134:2314-21.

Wells, J. G. , B. R. Davis, I. K. Wachsmuth, L. W. Riley, R. S. Remis, R. Sokolow, G. K. Morris. 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare Escherichia coli serotype. *J. Clin. Microbiol.* 18:512-20.

Yu, Liangli. 2008. *Wheat Antioxidants.* New Jersey: Wiley – Interscience Press.

APPENDIX-A

GROWTH CURVES OF *E. coli* O157:H7 IN THE PRESENCE OF PHENOLIC COMPOUNDS

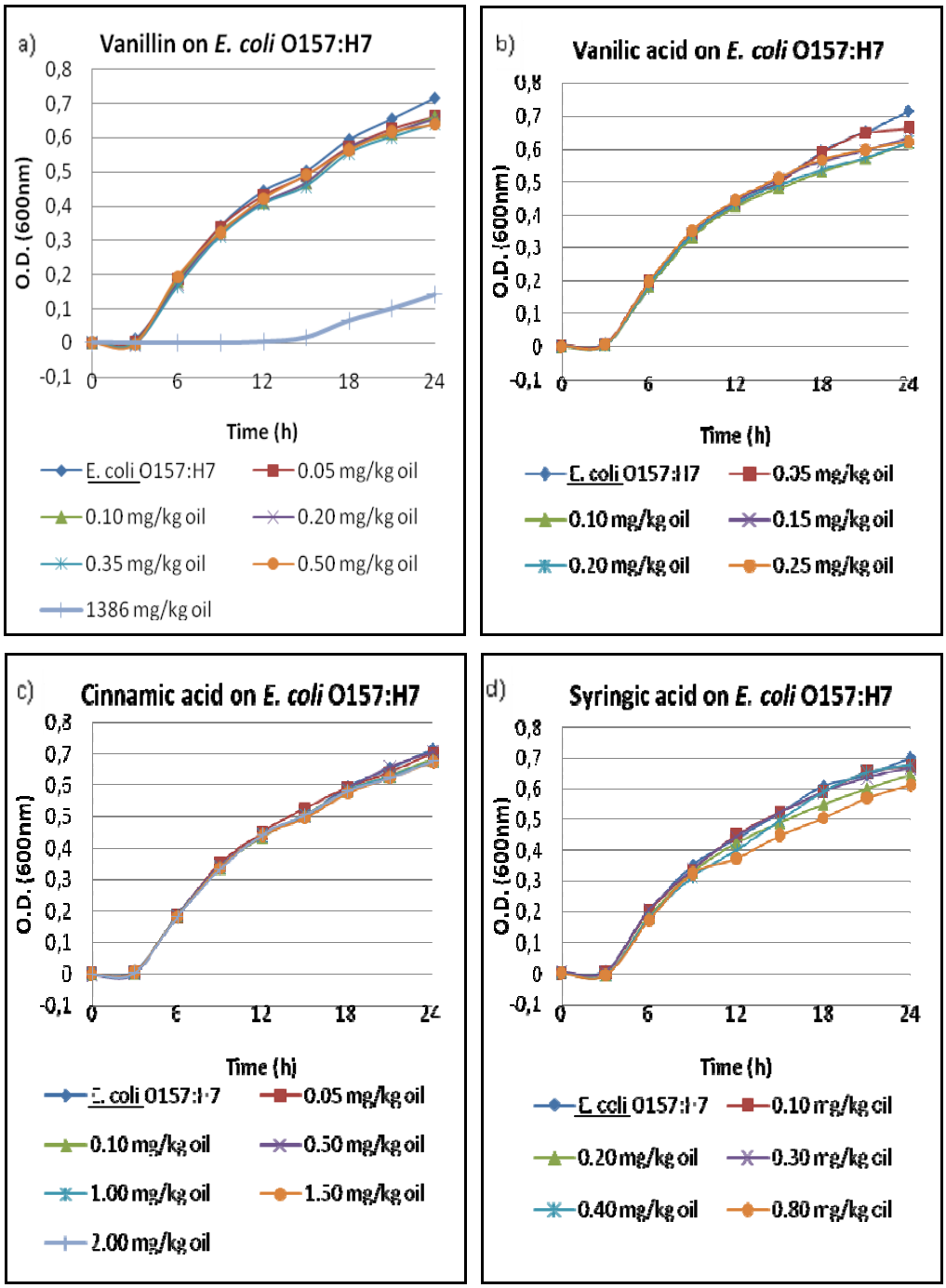

Figure A.1. The growth curves of *E. coli* O157:H7 in the presence of a) vanillin, b) vanillic acid, c) cinnamic acid and d) syringic acid. Standard deviation values are as follows; a) <0.03, b) <0.08, c) <0.02 and d) <0.01

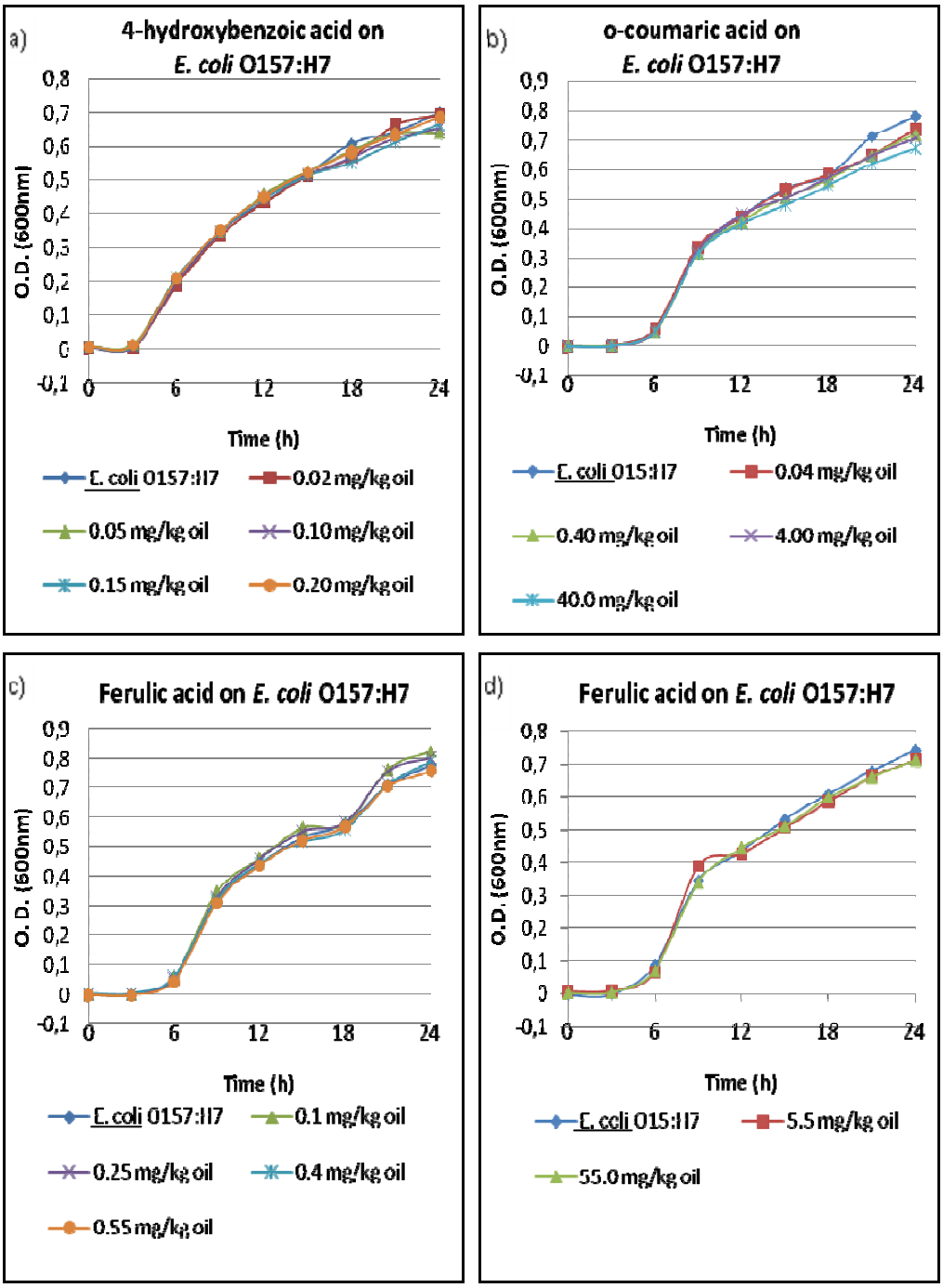

Figure A.2. The growth curves of *E. coli* O157:H7 in the presence of a) 4-hyrdoxybenzoic acid, b) o-coumaric acid, c) and d) ferulic acid. Standard deviation values are as follows; a) <0.07, b) <0.02, c) <0.01 and d) 0.02

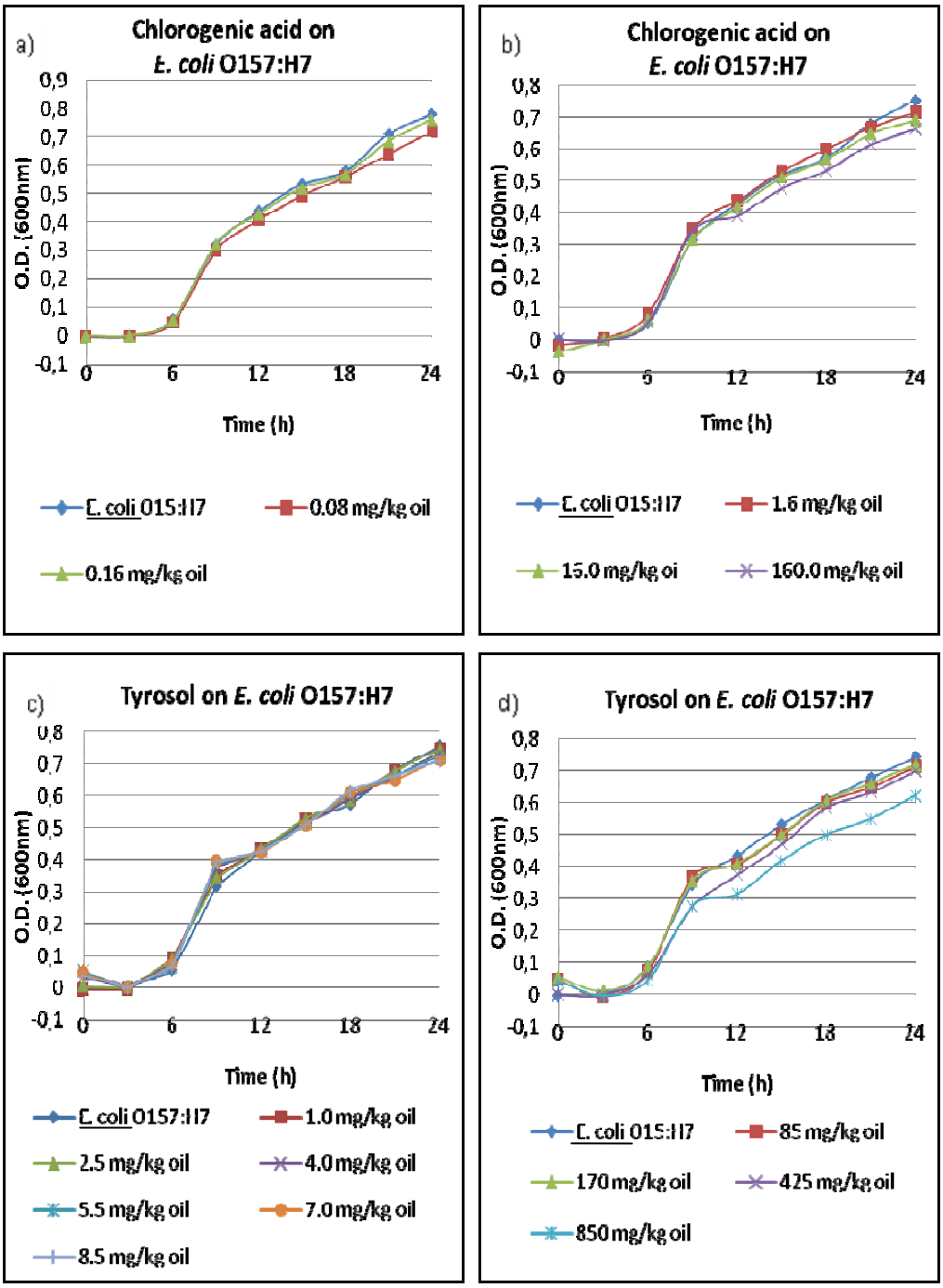

Figure A.3. The growth curves of *E. coli* O157:H7 in the presence of a) and b) chlorogenic acid, c) and d) tyrosol. Standard deviation values are as follows; a) <0.02, b) <0.02, c) <0.06 and d) 0.03

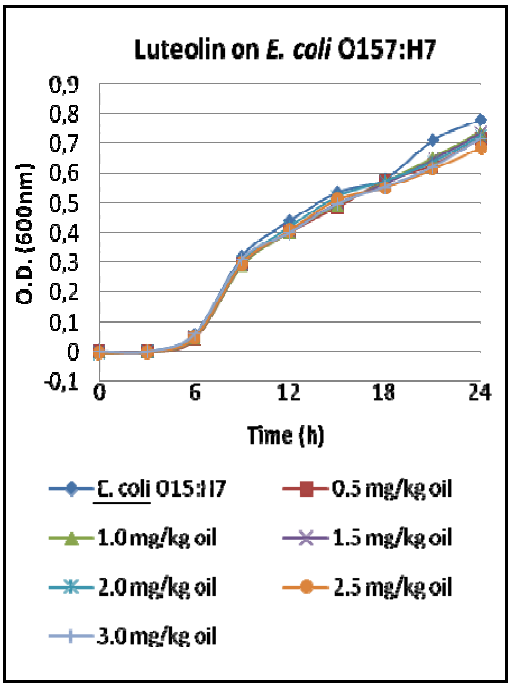

Figure A.4. The growth curves of *E. coli* O157:H7 in the presence of luteolin. Standard deviation values are lower than 0.08

APPENDIX-B

GROWTH CURVES OF *L. monocytogenes* IN THE PRESENCE OF PHENOLIC COMPOUNDS

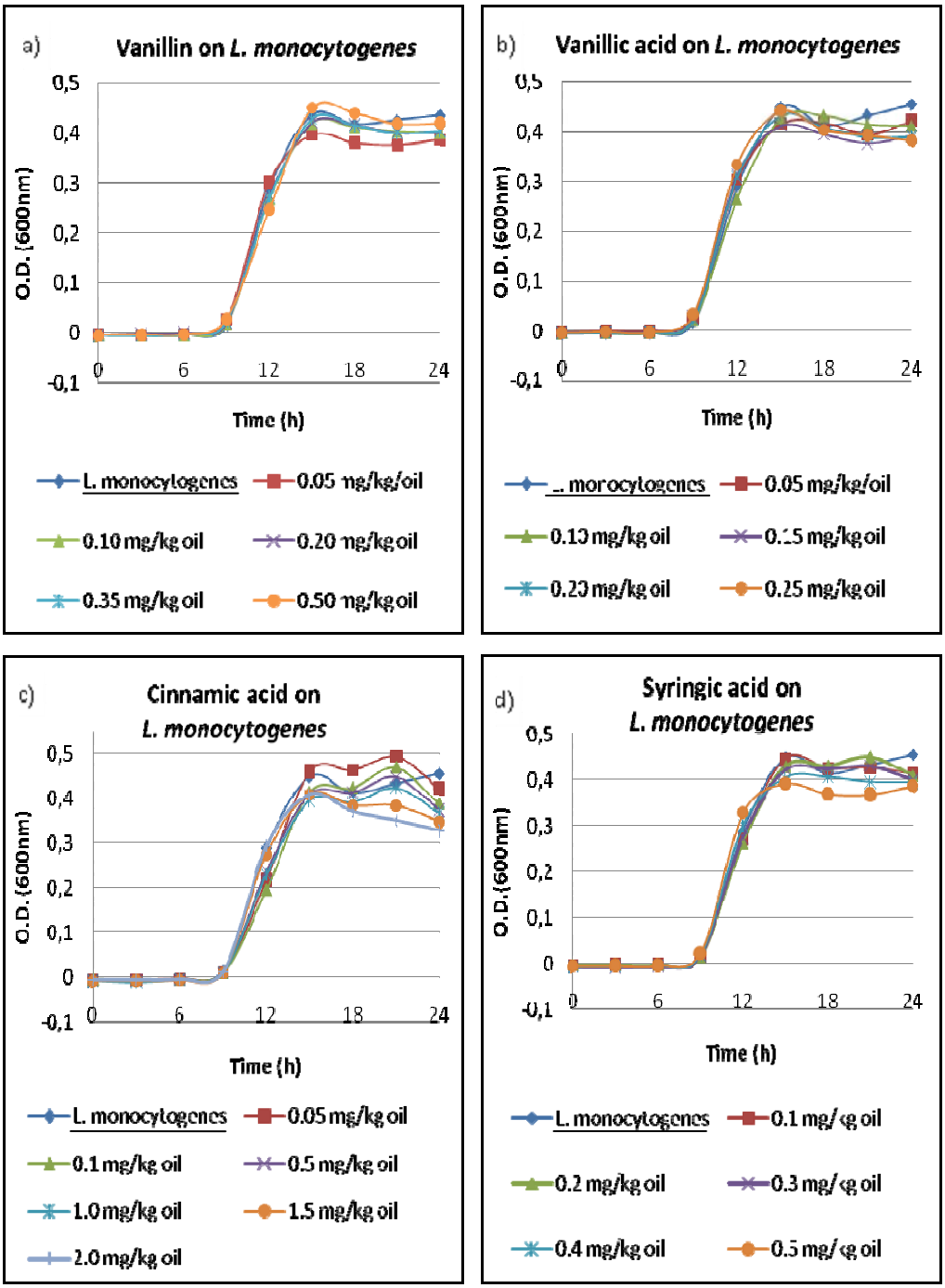

Figure B.1. The growth curves of *L. monocytogenes* in the presence of a) vanillin, b) vanillic acid, c) cinnamic acid and d) syringic acid. Standard deviation values are as follows; a) <0.003, b) <0.002, c) <0.01 and d) <0.002

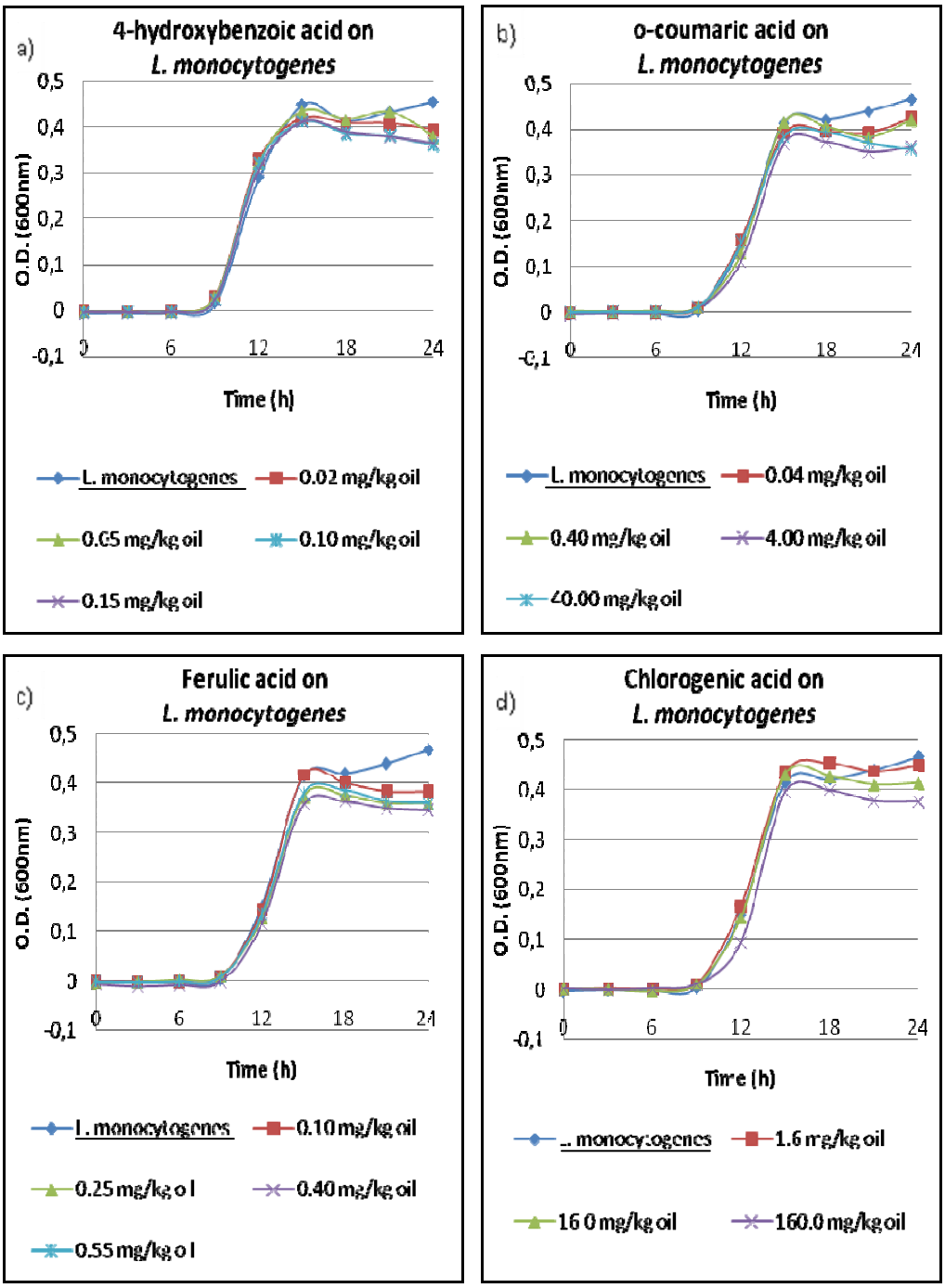

Figure B.2. The growth curves of *L. monocytogenes* in the presence of a) 4-hydroxybenzoic acid, b) o-coumaric acid, c) ferulic acid and d) chlorogenic acid. Standard deviation values are as follows; a) <0.07, b) <0.02, c) <0.01 and d) 0.02

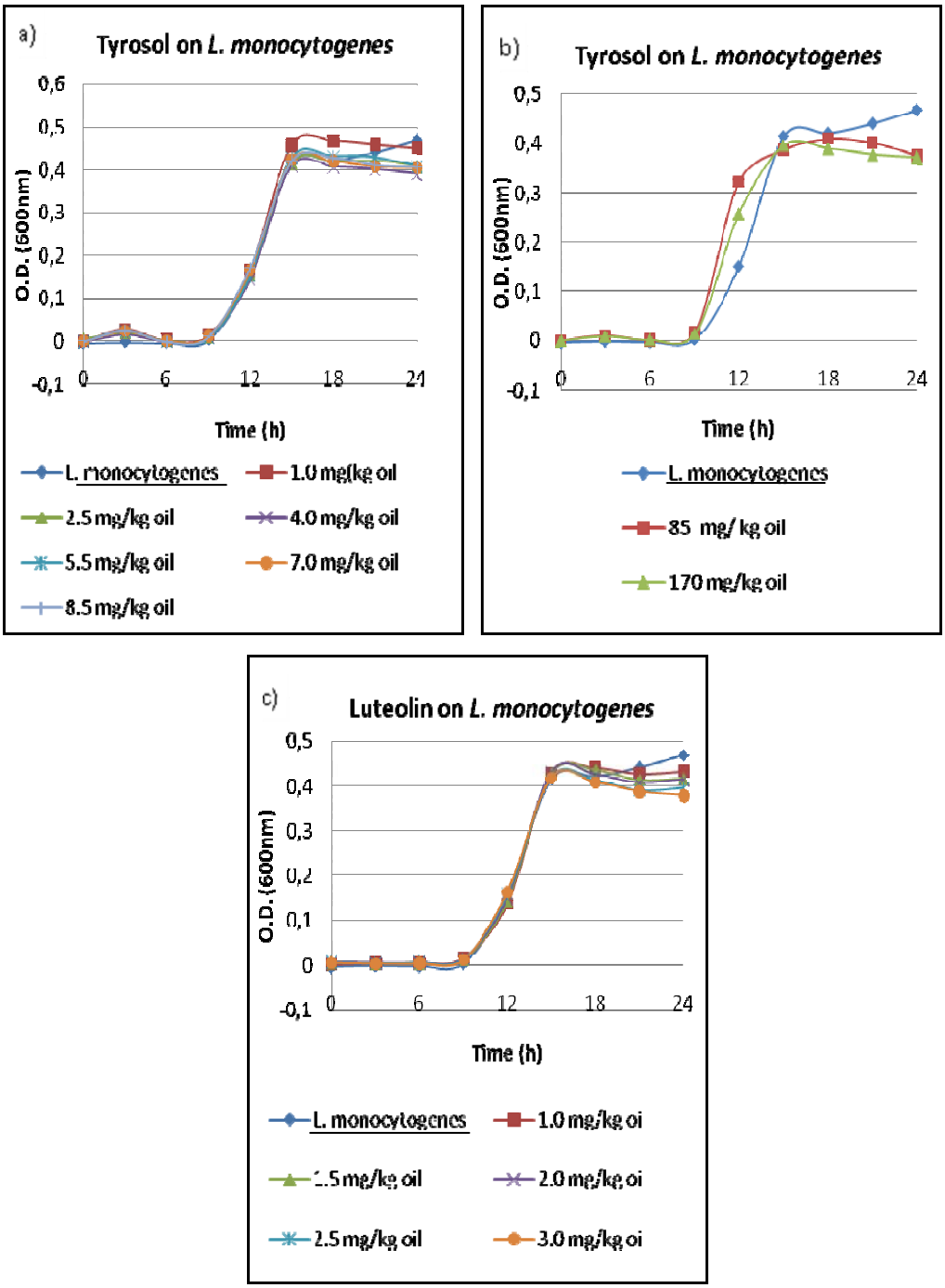

Figure B.3. The growth curves of *L. monocytogenes* in the presence of a) and b) tyrosol, and c) luteolin. Standard deviation values are as follows; a) <0.05, b) <0.31 and c) <0.006

APPENDIX-C

GROWTH CURVES OF *S.* ENTERITIDIS IN THE PRESENCE OF PHENOLIC COMPOUNDS

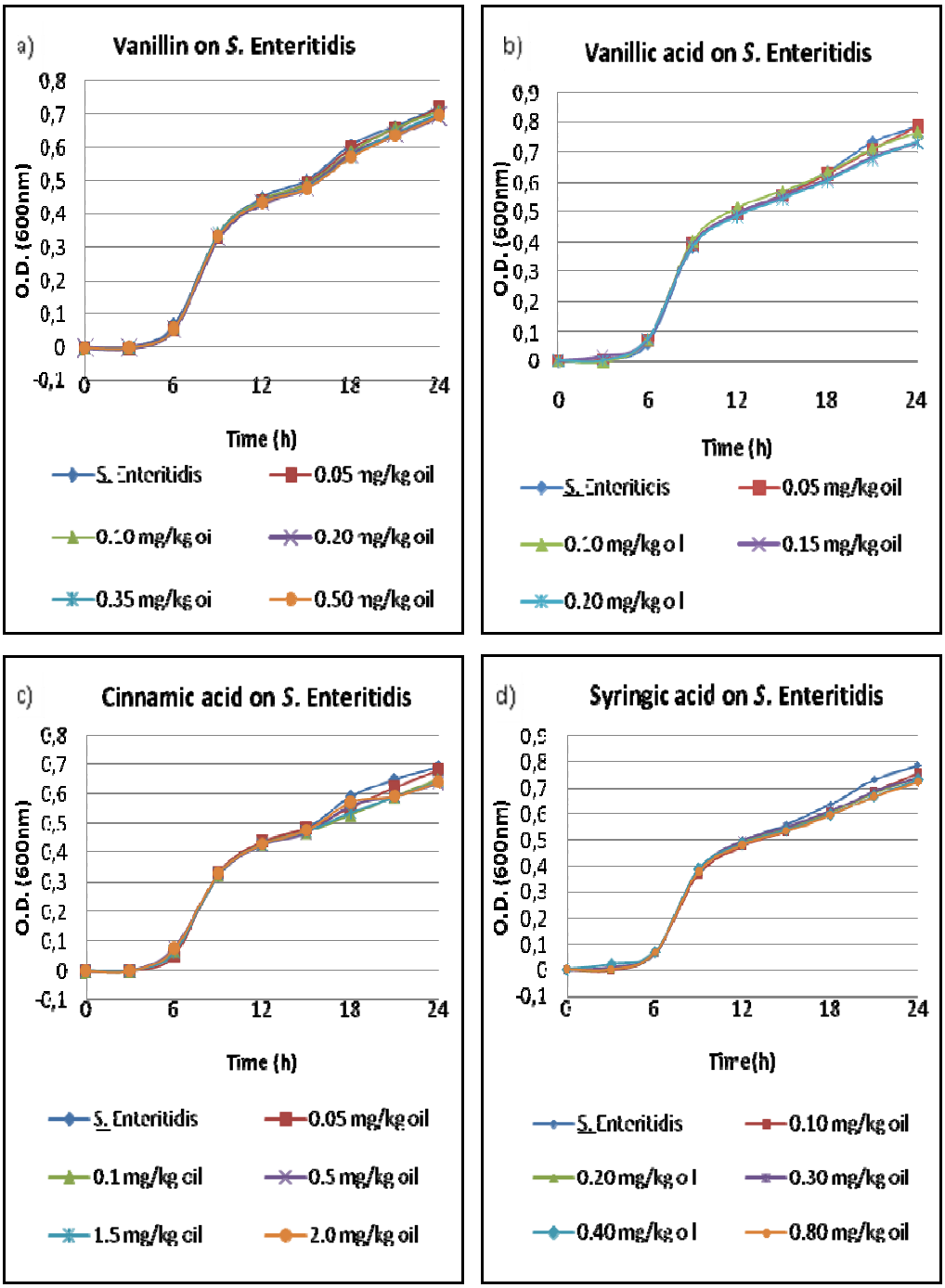

Figure C.1. The growth curves of *S.* Enteritidis in the presence of a) vanillin, b) vanillic acid, c) cinnamic acid and d) syringic acid. Standard deviation values are as follows; a) <0.02, b) <0.007, c) <0.02 and d) <0.03

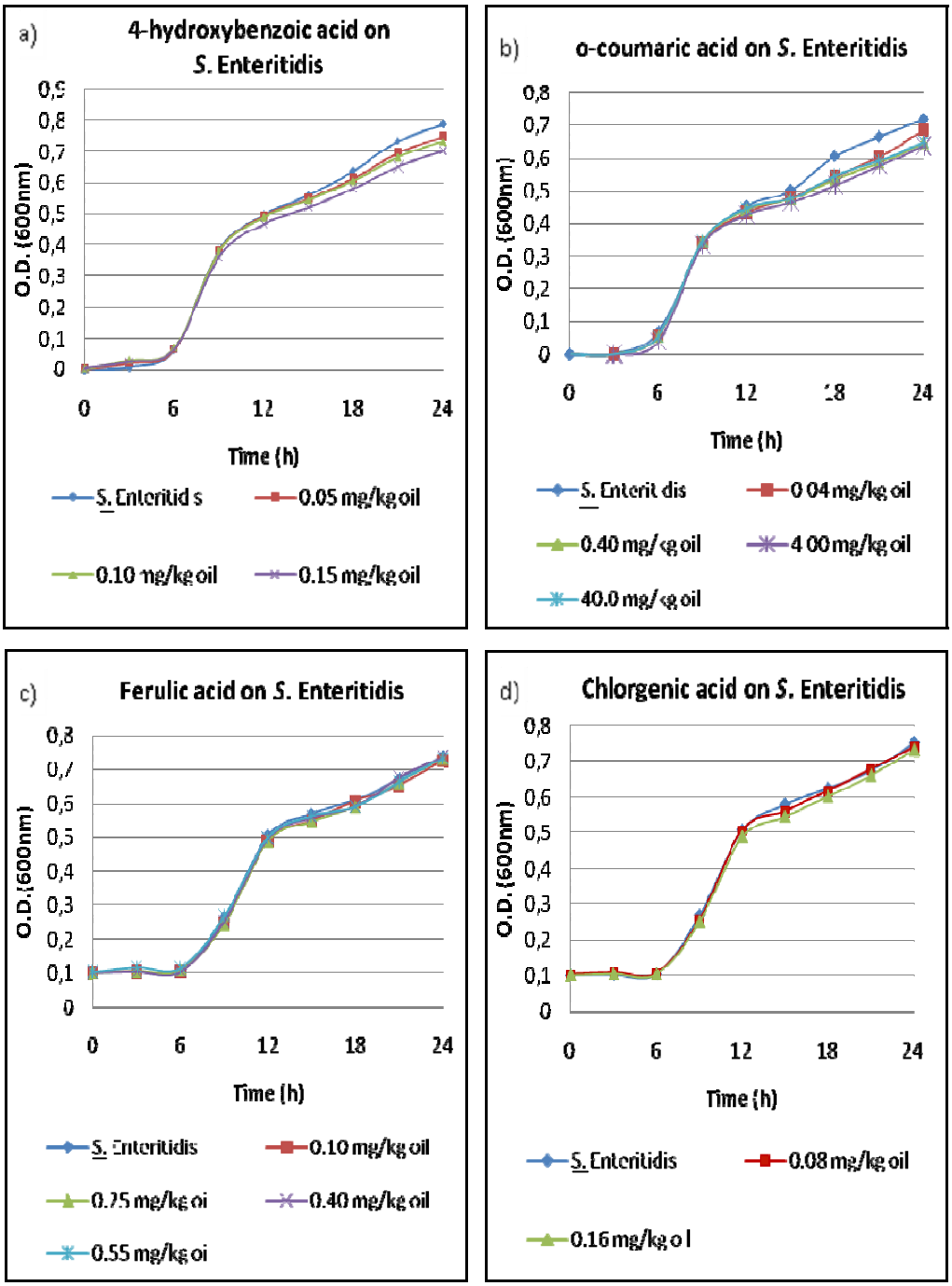

Figure C.2. The growth curves of *S.* Enteritidis in the presence of a) 4-hydroxybenzoic acid, b) o-coumaric acid, c) ferulic acid and d) chlorogenic acid. Standard deviation values are as follows; a) <0.02, b) <0.01, c) <0.01 and d) 0.01

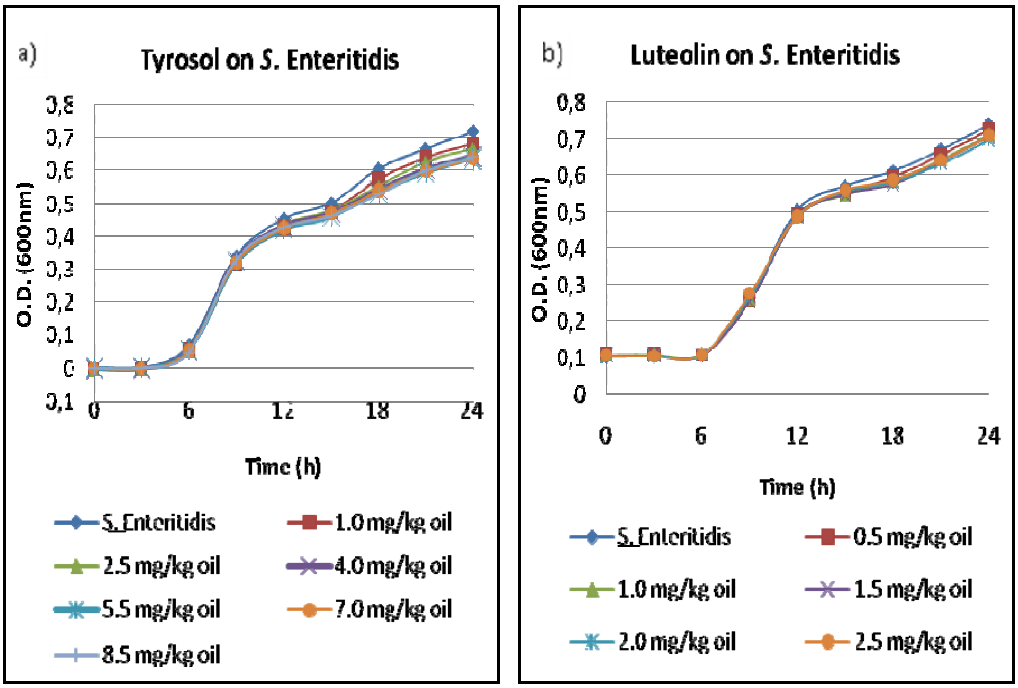

Figure C.3. The growth curves of *S.* Enteritidis in the presence of a) tyrosol and b) luteolin. Standard deviation values are as follows; a) <0.03 and b) <0.008

APPENDIX-D

EXPERIMENTAL DATA OF COMBINATIONAL ACTIVITY OF TYROSOL, VANILLIN, VANILLIC AND CINNAMIC ACIDS

Table D.1. Experimental data of combinational activity of tyrosol, vanillin, vanillic and cinnamic acids.

		Phenolic Compounds (mg/kg oil)				
Exp No	**Run Order**	**Vanillin**	**Vanillic acid**	**Cinnamic acid**	**Tyrosol**	**% Inhibition**
1	1	0.1	0.1	0.5	1	4.049059
2	31	1	0.1	0.5	1	-0.56387
3	40	0.1	1	0.5	1	4.311437
4	46	1	1	0.5	1	5.995054
5	16	0.1	0.1	3	1	3.653058
6	18	1	0.1	3	1	7.52296
7	43	0.1	1	3	1	3.087835
8	15	1	1	3	1	7.532531
9	34	0.1	0.1	0.5	10	2.405744
10	22	1	0.1	0.5	10	5.885275
11	23	0.1	1	0.5	10	4.507451
12	28	1	1	0.5	10	5.168512
13	8	0.1	0.1	3	10	5.242129
14	26	1	0.1	3	10	6.05622
15	10	0.1	1	3	10	-5.92518
16	30	1	1	3	10	-1.25129
17	47	0.55	0.55	1.75	5.5	2.541884
18	38	0.55	0.55	1.75	5.5	7.556142
19	4	0.1	0.1	0.5	1	4.003194
20	20	1	0.1	0.5	1	0.105541
21	13	0.1	1	0.5	1	9.075637
22	53	1	1	0.5	1	7.445005
23	21	0.1	0.1	3	1	8.48945
24	27	1	0.1	3	1	6.414369
25	35	0.1	1	3	1	8.916117

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Table D.1**.** (cont.). Experimental data of combinational activity of tyrosol, vanillin, vanillic and cinnamic acids

		Phenolic Compounds (mg/kg oil)				
Exp No	**Run Order**	**Vanillin**	**Vanillic acid**	**Cinnamic acid**	**Tyrosol**	**% Inhibition**
26	11	1	1	3	1	5.864379
27	51	0.1	0.1	0.5	10	10.69398
28	50	1	0.1	0.5	10	7.601909
29	17	0.1	1	0.5	10	10.68146
30	12	1	1	0.5	10	6.16994
31	9	0.1	0.1	3	10	11.13827
32	5	1	0.1	3	10	6.222727
33	54	0.1	1	3	10	1.212641
34	3	1	1	3	10	-1.89596
35	33	0.55	0.55	1.75	5.5	11.63001
36	14	0.55	0.55	1.75	5.5	1.91682
37	48	0.1	0.1	0.5	1	2.440417
38	36	1	0.1	0.5	1	-2.94603
39	37	0.1	1	0.5	1	8.863131
40	52	1	1	0.5	1	5.564447
41	41	0.1	0.1	3	1	8.934099
42	42	1	0.1	3	1	8.216078
43	44	0.1	1	3	1	9.188363
44	24	1	1	3	1	5.685188
45	45	0.1	0.1	0.5	10	9.749448
46	29	1	0.1	0.5	10	6.292138
47	32	0.1	1	0.5	10	9.44531
48	49	1	1	0.5	10	4.297562
49	6	0.1	0.1	3	10	9.920227
50	19	1	0.1	3	10	5.351975
51	7	0.1	1	3	10	1.212608
52	39	1	1	3	10	-3.33766
53	2	0.55	0.55	1.75	5.5	9.834258
54	25	0.55	0.55	1.75	5.5	7.675691
55	55	0.55	0.55	1.75	5.5	-1.78469
56	56	0.55	0.55	1.75	5.5	1.668251
57	57	0.55	0.55	1.75	5.5	4.001737

APPENDIX-E

GALLIC ACID STANDARD CURVE

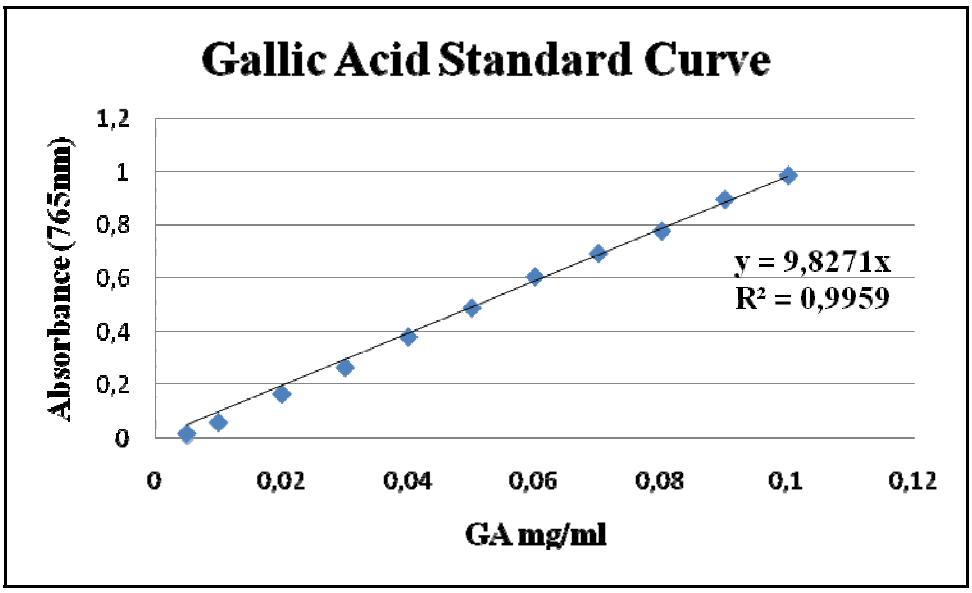

Figure E.1. The calibration curve using standard solution of gallic acid (0.005-1 mg/mL). Standard deviation values are lower than 0.003.

APPENDIX-F

PHENOLIC PROFILES OF EXTRA VIRGIN OLIVE OIL SAMPLES

Table F.1. Phenolic profiles and Total Phenolic Content (TPC) values of EVOO samples used in this study. 4-hydbn. acid, chlorog. acid, van. acid, syr acid, o-coum. acid, cinn. acid, hydxytyr., 2,3-dihydbe. acid, 4-hydphe. acid, 3-hydphe. acid, p-coum. acid, and m-coum. acid stand for 4-hyroxy- benzoic acid, chlorogenic acid, vanillic acid, syringic acid, o-coumaric acid, cinnamic acid, hydroxytyrosol, 2,3-dihydroxybenzoic acid, 4-hydroxyphenolic acid, 3-hydroxyphenolic acid, p-coumaric acid and m-coumaric acid, respectively (Source: Ocakoglu 2008)

	Phenolic Compound Concentrations (mg/kg oil)									
EVOO	***4-hydben. Acid**	***Tyrosol**	***Chlorog. acid**	***Van. acid**	***Vanilin**	***Syr. acid**	***Ferulic acid**	***O-coum. acid**	***Cinn. acid**	***Luteolin**
Erkence	0	5.316	0	0.089	0	0.065	0.036	0	1.975	0
Burhaniye	0.065	4.327	0	0.177	0.045	0.693	0.523	0.031	0.045	1.758
Koçarlı	0.124	8.589	0.076	0.119	0.021	0.128	0.171	0.039	0.465	2.140
Ödemiş	0.157	7.399	0.061	0.083	0.019	0.212	0.158	0	1.061	2.072
Dalaman	0.091	4.767	0	0.081	0	0.239	0.409	0	0.749	2.294
Gömeç	0.038	2.574	0	0.129	0.039	0.638	0.074	0	0.038	1.200
Altınoluk	0	0.825	0	0.128	0.022	0.302	0.050	0	0.016	0.181
Memecik	0.097	2.844	0	0.213	0	0.082	0.484	0	1.205	1.560
Nizip	0	1.434	0.157	0.038	0	0.052	0	0	0.090	0.126

*The phenolic compounds which are found in EVOO samples and are subjected to antimicrobial tests.

(Cont. on next page)

Table F.1. (Cont.) Phenolic profiles and Total Phenolic Content (TPC) values of EVOO samples used in this study. 4-hydbn. acid, chlorog. acid, van. acid, syr acid, o-coum. acid, cinn. acid, hydxytyr., 2,3-dihydbe. acid, 4-hydphe. acid, 3-hydphe. acid, p-coum. acid, and m-coum. acid stand for 4-hyroxy- benzoic acid, chlorogenic acid, vanillic acid, syringic acid, o-coumaric acid, cinnamic acid, hydroxytyrosol, 2,3-dihydroxybenzoic acid, 4-hydroxyphenolic acid, 3-hydroxyphenolic acid, p-coumaric acid and m-coumaric acid, respectively (Source: Ocakoglu 2008).

	Phenolic Compound Concentrations (mg/kg oil)									
EVOO	**Hydxytyr.**	**2,3- dihydbe. Acid**	**4 –Hydph. Acid**	**3-hydphe. Acid**	**Caffeic Acid**	**p-coum. acid**	**m-coum. Acid**	**Oleuropein**	**Apigenin**	**TPC**
Erkence	0.913	0	0	0	0	0.141	0	2.701	28.256	353.36
Burhaniye	10.406	1.197	0.407	0.735	0	0.862	0.051	9.553	6.322	342.93
Koçarlı	3.536	0	0.029	0	0.037	0.385	0.039	2.677	4.452	301.83
Ödemiş	4.963	0.423	0.226	0	0	0.620	0.050	5.267	9.466	291.07
Dalaman	2.073	0.282	0.061	0	0.031	0.356	0.037	4.719	8.130	277.99
Gömeç	6.268	0.527	0.037	0.182	0.089	0.280	0.021	6.632	4.910	265.29
Altınoluk	1.681	0	0.052	0.111	0	0.200	0.012	3.651	3.390	195.42
Memecik	0.594	0	0	0	0	1.836	0	3.067	13.873	156.97
Nizip	0.151	0.995	0.038	0	0.014	0.012	0	0.506	7.264	125.29