MICROWAVE ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM TOMATO AND SOUR CHERRY POMACES

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

MERİÇ ŞİMŞEK

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
FOOD ENGINEERING

MAY 2010

Approval of the thesis:

MICROWAVE ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM TOMATO AND SOUR CHERRY POMACES

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ABSTRACT

MICROWAVE ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM TOMATO AND SOUR CHERRY POMACES

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May 2010, 173 pages

The objective of this study was to compare microwave assisted extraction (MAE) and conventional extraction methods for the extraction of total phenolic compounds (TPC) from tomato and sour cherry pomace. Antiradical efficiency (AE) of the extracts and also the concentration of phenolic compounds were determined. In MAE, the effects of microwave power (400 and 700 W), solvent type (water, ethanol and ethanol-water mixture at 1:1 v/v), extraction time (8-20 min) and solvent to solid ratio (10, 20 and 30 ml/g) were studied to observe the change of TPC and AE of the extracts. Conventional extraction was conducted for 6 h using different solvent to solid ratios (10, 20 and 30 ml/g) and solvent types (water, ethanol and ethanol-water mixture at 1:1 v/v).

When the power increased from 400 W to 700 W, TPC and AE values increased. In MAE, maximum TPC and AE values were obtained at a solvent to solid ratio of 20 ml/g. The highest TPC and AE values were obtained when the ethanol-water mixture was used for both MAE and conventional extraction. The highest TPC and AE of tomato pomace extracts was determined as 3.76 mg gallic acid equivalent, GAE/g sample and 4.84 mg 1,1-diphenyl-2-picrylhydrazyl, DPPH/g sample, respectively in MAE using power level of 700 W, ethanol-water mixture, solvent to solid ratio of 20 ml/g and extraction time of 14 min. The major phenolic acids detected in tomato pomace extract were gentisic acid and vannilic acid. For sour cherry pomace extracts, the highest TPC and AE was found as 14.14 mg GAE/g sample and 28.32 mg DPPH/g sample, respectively in MAE using power level of 700 W, solvent to solid ratio of 20 ml/g, ethanol-water mixture and extraction time of 12 min. Epicatechin was found to be the major phenolic acid in sour cherry pomace. There was no significant difference in different extraction methods in terms of TPC. On the other hand, AE value and concentration of major phenolic acids of tomato and sour cherry pomace increased when MAE was used.

Keywords: Antiradical efficiency, microwave assisted extraction, phenolic, sour cherry, tomato.

ÖZ

DOMATES VE VİŞNE POSALARINDAN FENOLİK BİLEŞİKLERİN MİKRODALGA İLE ÖZÜTLENMESİ

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Mayıs 2010, 173 sayfa

Bu çalışmanın amacı, vişne ve domates posalarından toplam fenolik maddenin (TFM) özütlenmesinde mikrodalga yardımlı özütleme metodu ile konvansiyonel ekstraksiyon metodunun karşılaştırılmasıdır. Ayrıca, özütlerin antioksidan aktivitesi (AA) ve fenolik bileşiklerin konsantrasyonları da bulunmuştur. Mikrodalga ile özütlemede, mikrodalga gücünün (400 ve 700 W), çözücü çeşidinin (su, etanol ve etanol-su karışımı), ekstraksiyon süresinin (8-20 dak), çözücü ile katı madde oranının (10, 20 ve 30 ml/g) özütlerin TFM ve AA değişimine etkileri araştırılmıştır. Konvansiyonel ekstraksiyon, farklı çözücü ile katı madde oranları (10, 20 ve 30 ml/g) ve farklı çözücü tipleri (su, etanol ve etanol-su karışımı) kullanılarak 6 saatte gerçekleştirilmiştir.

Mikrodalga gücünün 400 W ‘dan 700 W ‘a artışıyla, toplam fenolik madde ve antioksidan aktivitesi değerlerinin arttığı görülmüştür. Mikrodalga yardımlı özütlemede, maksimum TFM ve AA değerleri 20 ml/g çözücü-katı oranında gözlemlenmiştir. En yüksek TFM ve AA değerleri, her iki ekstraksiyon metodunda da etanol-su karışımı kullanılarak elde edilmiştir. Domates posası özütlerinde en yüksek TFM ve AA mikrodalga ile özütlemede 700 W güç seviyesi, etanol-su karışımı, 20 ml/g çözücü-katı oranı ve 14 dakika özütleme süresi kullanılarak, sırasıyla 3.76 mg GAE/g numune ve 4.84 mg DPPH/g numune olarak bulunmuştur. Domates posası özütünde bulunan başlıca fenolik asitler gentisik asit ve vannilik asittir. Vişne posası özütleri için, en yüksek TFM ve AA, mikrodalga ile özütlemede 700 W mikrodalga gücü, 20 ml/g çözücü katı oranı, etanol-su karışımı ve 12 dakika özütleme süresi kullanılarak sırasıyla 14.14 mg GAE/g numune ve 28.32 mg DPPH/g numune olarak bulunmuştur. Epikateşin vişne posasında başlıca fenolik bileşik olarak görülmüştür. TFM açısından farklı ekstraksiyon metodları arasında önemli bir farklılık bulunmamıştır. Diğer yandan, AA değerleri ve ana fenolik asitlerin konsantrasyonları mikrodalga kullanıldığında artmıştır.

Anahtar kelimeler: Antioksidan aktivitesi, mikrodalga ile özütleme, fenolik, vişne, domates.

to my lovely nephew, ALP.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor Prof. Dr. Gülüm Şumnu and co-supervisor Prof. Dr. Serpil Şahin for their support, guidance, encouragement, critics and advices from the beginning throughout the construction of this thesis study.

I would also like to thank to İlkem Demirkesen and Burçin Güzel for their comments and suggestions for writing the thesis. I would like to also thank to my family and Sezin Teğmen for their supports all time.

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CHAPTER 1

INTRODUCTION

1.1. Types of Phenolic Compounds and Their Biosynthesis

Phenolic compounds can be defined as compounds possessing an aromatic ring bearing one or more hydroxyl substituents (Robards et al., 1999). Their chemical structure may vary from quite simple compounds like phenol to highly polymerized compounds (Bravo, 1998).

In plants, phenolic compounds occur primarily in their mono-glycosylated form (Shahidi and Naczk, 1995; Manach et al., 2004). Phenolic compounds can also be conjugated with aliphatic organic acids, amines, lipids, oligosaccharides or other substituents (Carotenuto et al., 1997; Bravo, 1998; Norbeak and Kondo, 1999; Lin et al., 2002).

The broad range of naturally occurring phenolic molecules is resulted from the differences in the structure complexity, conjugation, hydroxylation and methoxylation. Therefore, more than 8,000 phenolic compounds have been determined in plants (Harborne and Williams, 2000; Wrolstad, 2005), and they can be basically categorized into several classes as shown in Table 1.1 (Harborne, 1989; Harborne et al., 1999).

Table 1.1. The most important classes of phenolic compounds in plants (Adapted from Waterman and Mole, 1994)

Phenolic Class	**Carbon Skeleton**
simple phenols	C_6
hydroxybenzoic acids	C_6-C_1
phenylacetic acids	C_6-C_2
hydroxycinnamic acids, coumarins	C_6-C_3
Naphthoquinones	C_6-C_4
Xanthones	C_6-C_1-C_6
stilbenes, anthraquinones	C_6-C_2-C_6
Flavonoids	C_6-C_3-C_6
Tannins	$(C_6$-$C_1)_n$, $(C_6$-C_3-$C_6)_n$
Biflavonoids	$(C_6$-C_3-$C_6)_2$
Lignans	$(C_6$-$C_3)_2$
Lignins	$(C_6$-$C_3)_n$

The metabolic pathways responsible for phenolic compound biosynthesis are the shikimate and the acetate pathways. Certain phenolic compounds are biosynthesized by each of these pathways alone. However, the flavonoids, which are the largest phenolic class, are the products of mixed biosynthetic origin whereby they include within their structures the biogentic sub-units of both the shikimate and acetate pathways (Mann, 1987; Dewick, 2002). An overview of the key intermediates and pathways involved in producing phenolic metabolites is shown in Figure 1.1. Plant metabolism initiates with photosynthesis under ultraviolet light energy from the sun absorbed by chlorophyll to synthesize NADPH (nicotinamide adenine diphosphate) and ATP (adenosine triphosphate), which behave as reducing and activating reagents, respectively in metabolic reactions. In the following 'dark reaction' of photosynthesis, carbon dioxide is reduced to produce four-, five- six- and seven-carbon carbohydrates, including glucose.

The key precursor intermediates in the biosynthesis of phenolic compounds comprise shikimic acid, a central intermediate in the shikimate pathway, and acetyl coenzyme A (acetyl-CoA) which is the starting unit for the acetate pathway. Coenzyme A is an important cofactor required by enzymes to activate substrates for subsequent reaction (Mann, 1987). Natural products originating via the acetate pathway fall into the biosynthetic class of compounds known as polyketides. The biosynthetic pathway of shikimic acid starts with a coupling of phosphoenol pyruvate and D-erythrose-4-phosphate via a series of conversions to obtain shikimic acid and 3-dehydroshikimic acid (Dewick, 2002). The formation of phenolic compounds from the shikimic acid and the enzymes playing a role in these formations are presented in Figure 1.2.

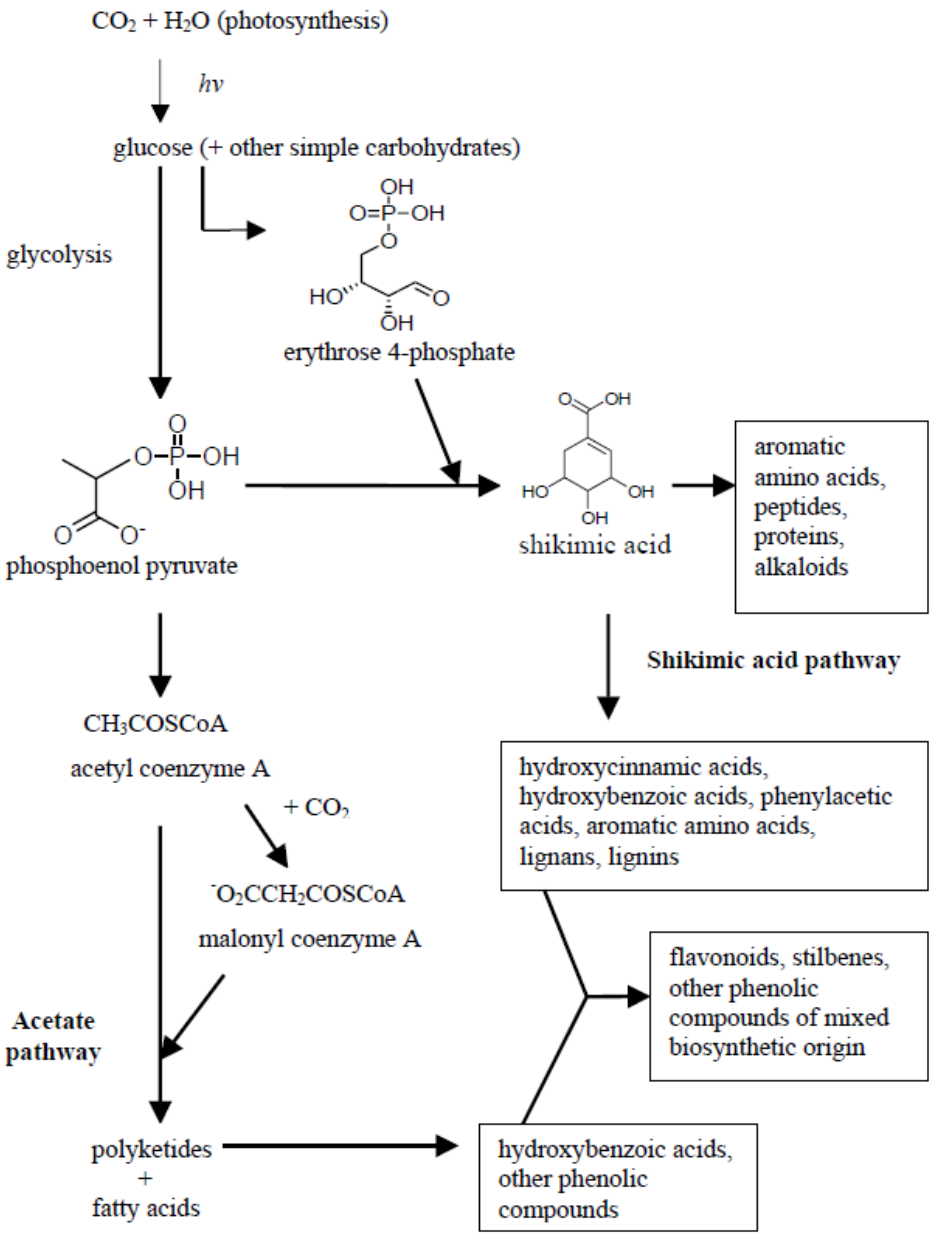


Figure 1.1. Overview of the biosynthesis of phenolic compounds (Adapted from Mann, 1987)

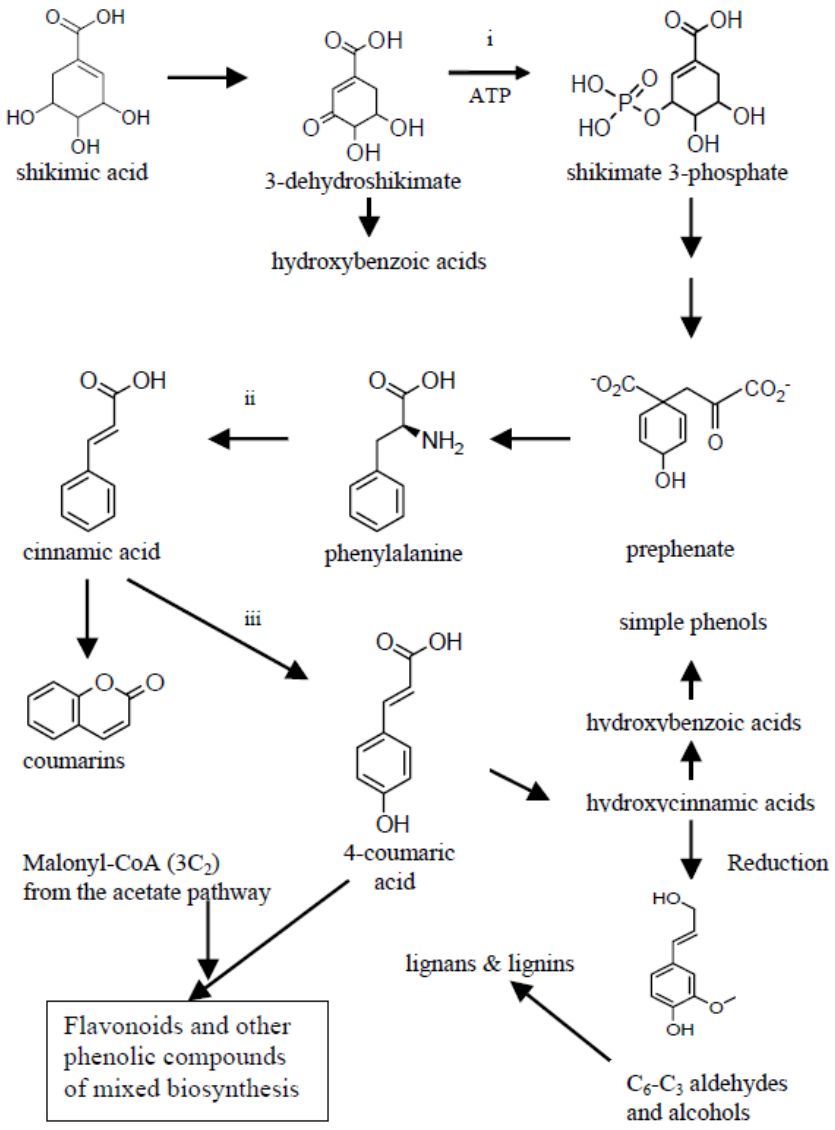
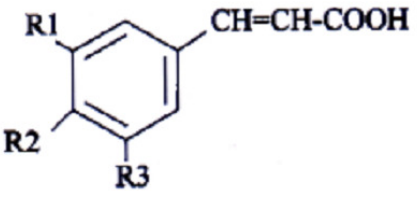


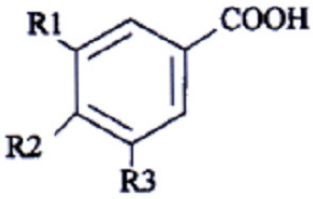
Figure 1.2. Formation of phenolic compounds from the shikimic acid pathway. Enzymes: i) shikimate kinase, ii) pheylalanine ammonia lyase (PAL), iii) cinnamic acid 4-hydroxylase (Adapted from Mann, 1987 and Dewick, 1993)

1.1.1. Phenolic acids

There are two main groups of phenolic acids, both of which are hydroxyl derivatives of aromatic carboxylic acids: hydroxybenzoic acids and hydroxycinnamic acids (Waksmundzka-Hajnos, 1998). Their derivatives differ in the patterns of

hydroxylation and methoxylation of their aromatic rings. Figure 1.3 shows the structures of these compounds.





R1	R2	R3	Compound
H	OH	H	*p*-Hydroxybenzoic acid
OH	OH	OH	Gallic acid
H	OH	OH	Protocatechuic scid

(a)

R1	R2	R3	Compound
H	OH	OH	Caffeic acid
CH_3O	OH	OH	Ferulic acid
H	OH	H	*p*-Coumaric acid

(b)

Figure 1.3. Basic structure of (a) hydroxbenzoic acid and (b) hydroxycinnamic acid derivatives (Adapted from Schuster and Hemann, 1985)

Hydroxybenzoic acids possess a general structure of C_6-C_1 (Figure 1.3a) (Balasundram et al., 2006). They are commonly found in bound form and rarely in the form of sugar derivatives. They include gallic, *p*-hyroxybenzoic, protocatechuic, vannilic and syringic acids. Generally, with the exception of black radish and onions, the hydroxybenzoic acid content of edible plants is very low (Manach et al., 2004). In various fruits or vegetables, the hydroxybenzoic acids are found as free acids such as gallic acid. Furthermore, the conjugation of gallic acid is present, its dimer, trimer and tetramer are ellagic acid, tergallic acid and gallagic acid, respectively. Although its trimer and tetramer are rarely present, gallic and ellagic acids are esterified to glucose in hydrolysable tannins. In addition, gallic acid is esterified to condensed tannins, their monomers, some derived tannins and quinic acid (Tomás-Barberán and Clifford, 2000).

Hydroxycinnamic acids are aromatic compounds with a three-carbon side chain (C_6-C_3) (Figure 1.3b). They are mainly present in bound form and are rarely found in the free form. The most common hydroxycinnamic acids are caffeic, ferulic, *p*-coumaric and sinapic acids (Meyer et al., 1998). Among these, caffeic acid is the predominant hydroxycinnamic acid in many fruits, and it represents over 75% of the total hydroxycinnamic acids in fruits. The most abundant phenolic acid present in cereal grains is ferulic acid held by plant cell walls. Also, the combination of caffeic and quinic acid forming chlorogenic acid is found as high amount in coffee, and lower in other plant foods (Kroon and Williams, 1999).

1.1.2. Flavonoids

Flavonoids represent the largest and the widely distributed group of plant phenolics. Their common structure is C_6-C_3-C_6 which is composed of two aromatic rings (A and B ring) linked through a three carbon bridge that is usually an oxygenated heterocycle (C ring). Figure 1.4 demonstrates the basic structure and the system used for the carbon numbering of the flavonoid nucleus. The various classes of flavonoids differ in the level of oxidation and pattern of substitution of the C ring, while individual compounds within a class differ in the pattern of substitution of the A and B rings. Flavonoids can be classified with six different subclasses which are flavonols, flavones, flavanones, flavanols, isoflavones and anthocyanins.

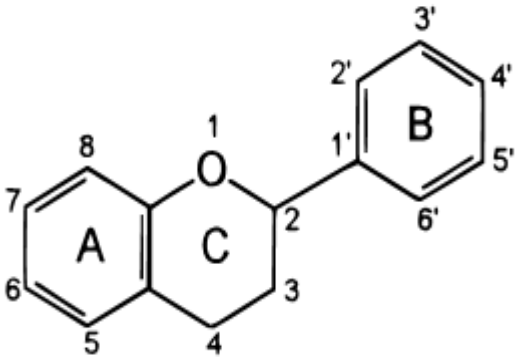


Figure 1.4. Basic structure of flavonoid skeleton (Adapted from Pietta, 2000).

1.1.2.1. Flavanols and Flavonols

Flavanols are known as flavan-3-ols (Figure 1.5a), and they are subunits of proanthocyanidins, which have a hydroxyl group attached to the position of the C ring, no positive charge on the oxygen atom and no double bond in the C ring.

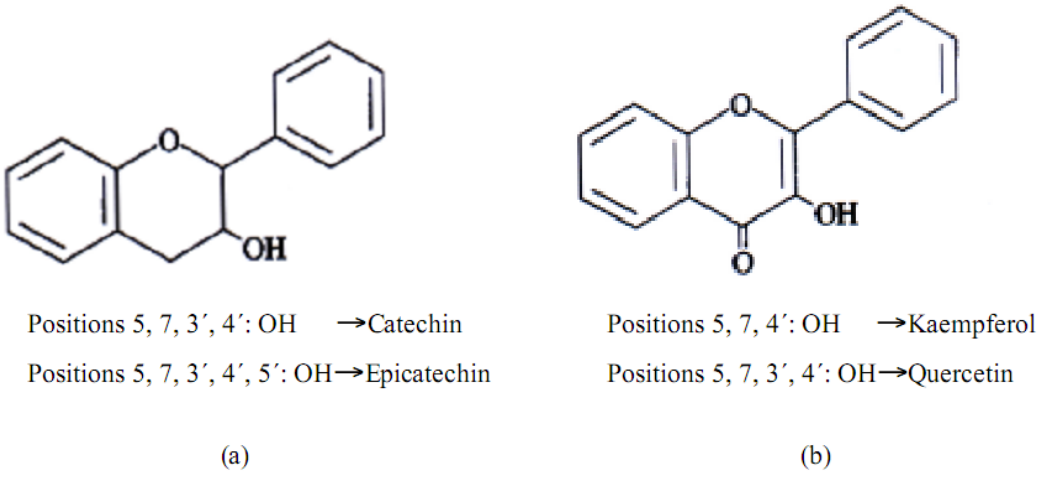


Figure 1.5. Basic structure of (a) flavanol and (b) flavonol skeletons (Adapted from Pietta, 2000).

Flavanols are found in the monomer form (catechins) as well as in the polymer form (proanthocyanidins). Also, they differ from other groups of flavonoids with the characteristic of not being glycosylated in foods. They are present in many types of fruits, red wine (up to 300 mg/L), green tea and chocolate. The amount of catechin can range up to 200 mg with the infusion of green tea. In addition, black tea comprises fewer amounts of monomer flavanols that are oxidized during fermentation of tea leaves to the more complex condensed polyphenols identified as theaflavins (dimers) and thearubigins (polymers) (Arts et al., 2000). Catechin and epicatechin are the main flavanols in fruit, but gallocatechin, epillocatechin, and epigallocatechin gallate are present in certain seeds of leguminous plants, in grapes and more importantly in tea (Manach et al., 2004). Proanthocyanidins, which are also known as condensed tannins, are dimers, oligomers, and polymers of catechins.

Furthermore, the estimation of the proanthocyanidin content of foods is hard due to possessing a wide range of structures and molecular weights (Manach et al., 2004). Condensed tannins are responsible for the astringent characteristic of fruit (grapes, peaches, kais, apples, pears, berries, etc.) and beverages (wine, cider, tea, beer, etc.), and for the bitterness of chocolate during the formation of complexes with salivary proteins (Santos-Buelga and Scalbert, 2000). The astringency is altered and vanishes with the maturity stage of fruits. This change has been explained in the kaki fruit with the polymerization reactions (Tanaka et al., 1994). Also, the significant decrease in tannin content, which is commonly observed through the maturation stage of many different kinds of fruits, is considered to be stemmed from the polymerization of tannins.

The structures of flavonols (Figure 1.5b) are very similar to the flavanols, except that it has a double-bonded oxygen atom attached to position 4 of the C ring and a double bond in the C ring. The flavonols are widely distributed in fruits; for example, quercetin, kaempferol, myricetin and isorhamnetin are predominant. Quercetin is abundant in fruits and vegetables, especially in onions. Quercetin conjugates are the main flavonols in onions found to be in the range of 11 to 29 mg/100 g fresh weight in the bulb of red onion cultivars (Bilyk et al., 1984). For example, strawberry, raspberry and blackberry are dominated by quercetin, kaempferol and their glycosides (Rommel and Wrolstad, 1993). In addition, apricots, plums and peaches contain significant amounts of kaempferol and quercetin (Bengoechea et al., 1997; Garcia et al., 1994 and Henning and Herrmann, 1980 a, b).

1.1.2.2. Flavanones, Flavones and Isoflavonoids

Flavanones (Figure 1.6a) and flavones (Figure 1.6b) have structures similar to those of flavanol and flavonols, respectively. However, in each case, there is no longer a hydroxyl group attached to the 3 position of the C ring.

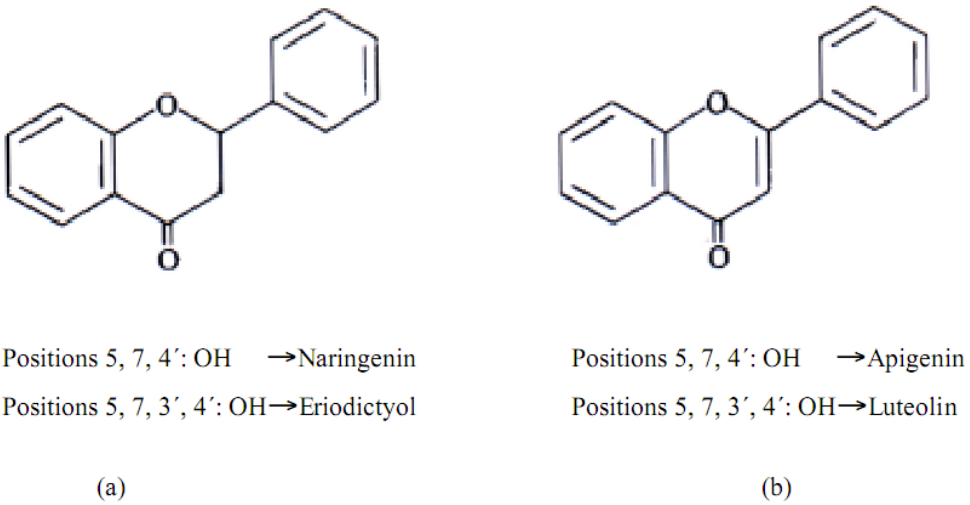


Figure 1.6. Basic structure of (a) flavanone and (b) flavones skeleton (Adapted from Pietta, 2000).

Flavanones are found in tomatoes and certain aromatic plants such as mint, but they are present only in citrus fruit at high concentrations. The main aglycones are naringenin in grapefruit, hesperetin in oranges, and eriodictyol in lemons. Flavanones are generally glycosylated by a disaccharide at position 7: either a neohesperidose, which imparts a bitter taste or a rutinose, which is favorless. Orange juice contains between 200 and 600 mg hesperidin/L and 15-85 mg narirutin/L. Moreover, a single glass of orange juice may contain between 40 and 140 mg flavanone glycosides (Manach et al., 2004).

Flavones consisting apigenin, luteolin and tricin are seen much less frequently than flavonols. For instance, apigenin and luteolin are present in cereal grains and aromatic herbs (parsley, apigenin and thyme) while their hydrogenated analogues hesperetin and naringin are almost exclusively found in citrus fruits (Pietta, 1999). The skin of citrus fruits contains large quantities of polymethoxylated flavones, tangeretin, nobiletin, and sinensetin (up to 6.5 g/L of essential oil of mandarin) participating in taste (Tomás-Barberán and Clifford, 2000). These polymethoxylated flavones are the most hydrophobic flavonoids.

Isoflavonoids are a distinct class of flavonoids with structural similarities to estrogens. The most common compounds of isoflavonoids are daidzein and genistein. They are mainly present in legumes. Soya and its processed products are the major source of daidzein and genistein which are also found in black beans, green split peas and clover sprouts. Soybeans contain between 580 and 3800 mg isoflavones/kg fresh wt, and soymilk contains between 30 and 175 mg/L (Manach et al., 2004).

1.1.2.3. Anthocyanins

Besides the flavonols and flavones, the following most abundant and widely scattered flavonoids are the anthocyanins. These compounds give most fruits their red, violet and blue color (Hong and Wrolstad, 1986; Rommel et al., 1992) although the red color of some fruits (e.g. orange and tomato) is caused by carotenoid pigments rather than anthocyanins. They are found in various chemical forms, and in both colored and uncolored. Even though they are present in the aglycone (anthocyanidins) form in the plants; surprisingly, they are resistant to light, pH, and oxidation conditions tending to degrade them. The glycosylation and esterificiation as well the formation of complexes prevent the degradation of anthocyanins (Manach et al., 2004). They are found in fruits, leafy root vegetables (aubergines, cabbage, onions, beans and radishes), cereals and red wine. Anthocyanin in fruits is dependent on the color intensity and could reach up to 2-4 g/kg fresh weight in blackcurrants or blackberries (Manach et al., 2004). In addition, fruit anthocyanin content usually increases as the fruit matures (Pierpoint, 1986). Wine contains 200 mg anthocyanins/ L, and these anthocyanins are converted into different complex structures with the age of wine (Clifford, 2000).

1.1.2.4. Lignans and Stillbenes

Lignans are formed of 2 phenylpropane units. The linseed, which comprises secoisolariciresinol (up to 3.7 g/kg dry wt.) and low amount of matairesinolis, is the richest source of lignans. Also, scarce amounts of same lignans are found in other cereals, grains, fruit, and certain vegetables (Manach et al., 2004).

Stillbenes are present in low quantities in the human diet. The best known compound is resveratrol whose anticarcinogenic effects have been shown during screening of medicinal plants (Bertelli et al., 1998).

1.2. Phenolic Compounds in Foods of Plant Origin

Generally, all foods originated from plants comprise phenolic compounds, but the majority of human diet involves fruits, vegetables and beverages (Balasundram et al., 2006). Although many sources were investigated in regard to the phenolic compounds, these compounds still attract the attention of many researchers.

The study of Rop et al. (2009) concentrated on the determination of total phenolic content and antioxidant activity of different cultivars of the plum. The total phenolic content ranged from 2.27 to 4.95 mg of gallic acid/g fresh mass. The total antioxidant activity determined by ABTS method varied between 4.68 ± 0.21 mg ascorbic acid equivalents (AAE)/g fresh mass and 6.04 ± 0.21 mg AAE/g fresh mass.

Cevallos-Casals et al. (2006) investigated the total phenolic content and antioxidant activity of fourteen red-fleshed plum and eight peach genotypes. According to their results, total phenolic content varied from 298 to 563 mg chlorogenic acid equivalent (CGA)/100 g for plums and 100 to 449 mg CGA/100 g for peaches. For the antioxidant activity determined by DPPH method, the obtained relative antiradical capacity (RAC) values ranged from 1254 to 3244 µg trolox/g for plums and from 440 to 1784 µg trolox/ g for peaches.

In the study of Mansouri et al. (2005), total phenolic content and antioxidant activity of seven different varieties of ripe date palm fruit were determined. Total phenolic content was in the range of 2.49 ± 0.01 and 8.36 ± 0.60 mg gallic acid equivalents (GAE)/100 g fresh fruit. The fruit showed an antioxidant activity with the values of antiradical efficiency (AE) from 0.08 to 0.22.

In the study of Tanrioven and Eksi (2005), the phenolic contents of pear juice samples obtained from seven different varieties were analyzed by HPLC. The results showed that chlorogenic acid varied from 73.1 to 249 mg/L, epicatechin from 11.9 to

81.3 mg/L, caffeic acid from 2.4 to 11.4 mg/L and *p*-coumaric acid from 0.0 to 3.0 mg/L. In addition, total amount of polyphenol of pear juice samples ranged between 196 to 457 mg/L.

In the study of Gorinstein et al. (2004), total phenols and phenolic acids in the pulp of Jaffa sweeties and white grapefruits were determined using Folin- Ciocalteu reagent and HPLC analysis, respectively. Total polyphenols (μmol g^{-1} fresh weight) in peeled sweeties and grapefruit were 9.2 ± 0.9 and 7.0 ± 0.9 and in peels of sweeties and grapefruits were 13.9 ± 1.1 and 8.4 ± 0.9, respectively. In addition, ferulic acid was the predominant component, followed by *p*-coumaric, sinapic and caffeic acid. Total concentration (nmol/g) was higher in pulp (362) and peel (1513) of grapefruit than in sweeties (272 and 1277, respectively) for four of the hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic and sinapic).

Koca and Karadeniz (2009) examined the total phenolic content and antioxidant activity as ferric reducing antioxidant power (FRAP) of seven wild and ten cultivated blackberries, and six lowblush and four highblush blueberries. The total phenolic content of tested samples was found as 0.95-1.97 and 1.73-3.79 mg/g for blackberries, 0.18-2.94 and 0.77-5.42 mg/g for blueberries. In addition, FRAP values varied from 35.05 to 70.41 μmol/g for blackberries and 7.41 to 57.92 μmol/g for blueberries.

Red wine contain more than 1000 mg gallic acid equivalents (GAE)/L of total phenolics, compared to less than 500 mg GAE/L for most white wines (Mazza et al., 1999). In addition, Heinonen et al. (1998) reported that wines made from fruits or berries other than grapes were found to have lower total phenolic content than red wines. However, wines made from blueberries were found to have higher total phenolics content (600-1860 mg GAE/L) than that of white wines (191-306 mg GAE/L) (Sánchez- Moreno et al., 2003).

Several researches have been focused on the total polyphenol content in tea leaves and its extracts. Total polyphenol content in black and green tea leaves measured by

Khokhar and Magnusdottir (2002) was 80.5-134.9 mg/g and 65.8 and 106.2 mg/g, respectively. Hoff and Singleton (1977) estimated similar levels of polyphenols in green and black tea leaves. However, Manzocco et al. (1998) found higher polyphenol content in green tea leaves (94.5 mg/g) than in black tea leaves (80.1 mg/g).

Poyrazoglu et al. (2002) studied the determination of phenolic compounds in freshly prepared pomegranate juices obtained using thirteen pomegranate varieties from different growing regions of Turkey. They detected minor amounts of phenolic compounds in pomegranates as phenolic acids (gallic, protocatechuic, chlorogenic, caffeic, ferulic, *o*- and *p*-coumeric acids) and flavonoids (catechin, quercetin and phloridzin). On average, gallic acid was predominant with an overall mean concentration of 4.55 ± 8.55 mg/L. Catechin was the second most abundant with 3.72 ± 2.29 mg/L, followed by quercetin, chlorogenic acid, phloridzin, protocatechuic acid, caffeic acid, *o*-coumaric acid, *p*-coumaric and ferulic acid.

Mirdehghan and Rahemi (2007) investigated the total phenolic compound in arils and peel of pomegranate fruit during a period, starting 10 days after full bloom until harvesting. Total phenolics levels increased at early stage of growth both in peel and arils of fruit, but generally decreased during maturation and reached to 3.70 and 50.22 mg g^{-1} of dry weight in arils and peel, respectively at harvest.

Leantowicz et al. (2002) determined the total phenolic content of apples, peaches and pears. The content of total polyphenols (g/100 g) was 0.23 ± 0.03, 0.22 ± 0.03 and 0.68 ± 0.1 in peeled fruits and 0.48 ± 0.04, 0.47 ± 0.04 and 1.2 ± 0.12 in peels of peaches, pears and apples. Among the phenolic acids, caffeic, *p*-coumaric, ferulic were found in a higher amount in peeled apples than in peaches and pears, respectively.

Sun et al. (2002) reported that the phenolic content of apple (296 mg GAE/100 g), followed by red grape (201 mg/100 g, pineapple (94 mg/100 g), banana (90 mg/100

g), peach (84 mg/100 g), lemon (82 mg/100 g), orange (81 mg/100 g), pear (71 mg/100 g) and grapefruit (50 mg/100 g).

Gorinstein et al. (2001) reported that peeled lemons, oranges, and grapefruit contain 164 ± 10.3, 154 ± 10.2, and 135 ± 10.1, and their peels 190 ± 10.6, 179 ± 10.5, and 155 ± 10.3 mg GAE/100 g of total polyphenols, respectively.

Velioglu et al. (1998) investigated total phenolics of twenty-eight plant products, including sunflower seeds, flaxseeds, wheat germ, buckwheat, and several fruits, vegetables, and medicinal plants. The total phenolic content, determined according to the Folin- Ciocalteu method, varied from 169 to 10548 mg/100 g dry product.

Vegetables can be considered as an important source of dietary phenolic compounds; however, there are fewer studies related to the phenolic content of vegetables in comparison to fruits. The total phenolic content of carrot is reported to range from 509 to 779 mg chlorogenic acid equivalents/100 g dry weight (Talcott et al., 1999). In addition, spinach leaf comprises 162 to 483 mg chlorogenic acid equivalents/100 g fresh weight (Howard et al., 2002).

Toor and Savage (2005) observed that skin of all tomato cultivars had significantly higher levels of total phenolics, total flavonoids, lycopene, ascorbic acid, and antioxidant activity (both in hyrophilic and lipophilic extracts) when compared to their pulp and seed fractions. On average, the skin and seeds of the three cultivars in each fraction provided 53% of the total phenolic content, 52% of the total flavonoids, 48% of the total lycopene, 43% of the total ascorbic acid and 52 % of the total antioxidant activity, calculated on the basis of their actual fresh weights in whole tomato. These results indicate that the removal of skin and seeds of tomato during home cooking and processing lead to a significant decrease of all the major antioxidants. Therefore, the consumption of tomatoes with their skin and seeds is important to accomplish health benefits. The hydrophilic phenolics in the skin of three cultivars ranged from 26.9-30.3 mg GAE/100 g. George et al. (2004) found that the phenolic content in the skin of different tomato genotypes varied from 10-40 mg

catechin equivalents/100 g, and it varied in the pulp from 9-27 mg catechin equivalents/ 100g.

Valverde et al. (2002) examined nine commercial varieties of tomato produced in Spain for their lycopene content, phenolic compounds content and antioxidant activity. The phenolic compounds were identified as flavonoids (quercetin, kaempferol and naringenin) and hydroxycinnamic acids (caffeic, chlorogenic, ferulic and *p*-coumaric acids). The concentrations of the various phenolic compounds as well as the antioxidant activity were significantly affected by the tomato variety. Quercetin was the most abundant flavonoid, varying between 7.19 and 43.59 mg/kg fresh weight.

Stewart et al. (2000) observed the distribution of flavonols in Spanish cherry tomatoes. They showed that more than 98% of total flavonols occurred in the skin with the amount of 143.3 mg/kg fresh weight, followed by the seed with 1.5 mg/kg fresh weight, followed by the flesh with 0.12 mg/kg fresh weight.

Cherries are known to have anthocyanins as the major phenolics. Sour cherries are known to comprise various anthocyanins such as cyaniding 3-sophoroside, cyaniding 3-glucosylrutinoside, cyaniding 3-glucoside, and cyaniding 3-rutinoside (Chaovanalikit and Wrolstad, 2004; Chandra et al., 1992). It was also reported that sour cherries had cyaniding 3-arabinosylrutinoside, pelargonidin 3-glucoside, and peonidin 3-rutinoside (Chandra et al., 1992; Chandra et al., 2001). Among hydroxycinnamates, sour cherry has neochlorogenic acid and p-coumaroylquinic acid as the predominant compounds (Goncalves et al., 2004; Chaovanalikit and Wrolstad, 2004; Gao and Mazza, 1995). Flavonols and flavan-3-ols were also found in sweet and sour cherries, which included catechin, epicatechin, quercetin 3-glucoside, quercetin 3-rutinoside, and kaempferol 3-rutinoside (Goncalves et al., 2004; Chaovanalikit and Wrolstad, 2004; Gao and Mazza, 1995). Total phenolics of different sour cherry or sweet cherry were investigated by various researchers (Kim and Padilla-Zakour, 2004; Usenik et al., 2007) and found to change with cultivar.

Chaovanalikit and Wrolstad (2004) examined total anthocyanins, total phenolics, and the antioxidant activities of different parts of a sour cherry. Total phenolics were the highest in skins, intermediate in flesh, and lowest in pits.

1.3. Effect of Processing on Phenolic Compounds

Processed products show lower nutritional value than the fresh ones due to the loss of nutritional compounds such as phenolic compounds during processing (Burg and Fraile, 1995; Lathrop and Leung, 1980; Murcia et al., 2000; Rao et al., 1981). However, Stahl and Sies (1992) observed higher content of lycopene in a heat-processed tomato juice than that in unprocessed one. Similarly, Wang et al. (1996) found that heat processed tomato juice showed much higher antioxidant activity than the fresh ones. In addition, higher antioxidant activity was observed during thermal treatments such as steaming, microwaving and frying of the tomato fruits by Chen et al., (2000). As seen in the above mentioned studies, the use of various kinds of processing might increase the nutritional value of tomato.

In the study of Chang et al. (2006), two varieties of tomatoes were used to observe the effects of different drying processes, freeze-dried and hot-air-dried, on the antioxidant properties of tomatoes. The fresh tomatoes comprised the lowest total phenolic content, compared to processed ones. The increase in the amounts of total phenolic content was larger in hot air dried tomatoes as compared to freeze dried ones. This could be explained with the liberation of phenolic compounds from the matrix during the process. Similarly, processed tomatoes had higher total flavonoids as compared to fresh ones. This increase could be contributed with biochemical reactions occurred in flesh and peels of tomato during the drying process.

In the study of Gahler et al. (2003), the polyphenol content and the hydrophilic antioxidant activity were investigated in the tomato juice, baked tomatoes, tomato sauce, and tomato soup. No significant changes of the free phenolics were observed during the processing of tomato juice; however, the sum of free and bound phenolics changed considerably after four production steps (sieving, homogenization,

sterilization and bottling). Both free and total phenolic contents of tomatoes increased during baking. There were significant differences between baking temperatures in affecting the phenolic contents. The other tomato products exhibited a similar trend. These studies assisted the idea of increase of bioavailability of individual antioxidants due to their releasing from the matrix when tomato was processed.

1.4. Antioxidant and Medicinal Properties of Phenolic Compounds

Free radicals are known as reactive substances that have an unpaired electron, and they can be found as anionic, cationic or neutral. As they possess an unpaired electron, they tend to charge, and harm body cells to acquire the missing electron they need. Free radicals are chiefly obtained from reactive oxygen species (ROS) and reactive nitrogen species (Devasagayam et al., 2004). The term ROS is commonly used for giving oxygen-comprising molecules which are more reactive than triplet state oxygen in air (Noguchi and Niki, 1998). They can be produced as a result of normal metabolic processes or by various exogenous factors such as exposure to X-rays, ozone, cigarette smoke, car exhaust, air pollutants and industrial chemicals (Punchard and Kelly, 1996; Bagchi and Puri, 1998). In a normal human metabolism, the oxidant and antioxidant levels are retained in balance being crucial for maintaining optimal physiological conditions (Temple, 2000 and Thompson, 1994). Excess production of oxidants in certain conditions could result in an imbalance, and being in a state of oxidative stress causing to oxidative damage to large biomolecules such as lipids, DNA, and proteins (Liu, 2004). Therefore, they play a role in a host of serious diseases such as cancer, cardiovascular disease, Alzheimer's disease, aging, cataracts and macular degeneration (Devasagayam et al., 2004). The inhibition or delay of the biomolecules oxidation can be provided by blocking the initiation or propagation of oxidizing chain reactions (Valverde et al., 2002).

1.4.1. Antioxidant Mechanism of Phenolic Compounds

There are two main classes of antioxidants, namely, synthetic and natural. While synthetic antioxidants are compounds with phenolic structures of various degrees of

alkyl substitution, natural antioxidants can be phenolic compounds like tocopherols, flavonoids, and phenolic acids or carotenoids. Synthetic antioxidants such as butylated hdroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants. However, since the safety of synthetic antioxidants have been analyzed by elaborated and costly analyses (Pokorny, 2007), and carcinogen suspect of these compounds is present, the interest to find natural, cheap and safe sources for natural antioxidants (Barlow, 1990) has increased. Therefore, the investigations have been focused on the antioxidant compounds found in a number of plant sources in order to replace the synthetic antioxidants with the natural ones.

The antioxidant activity of phenolic compounds can be classified according to their action mechanisms as in the following: (i) Primary antioxidants are capable of stabilizing or detoxifying free radicals by donating hydrogen or electrons to them before they attack cells. (ii) Synergistic antioxidants are named as oxygen scavengers and chelators of metal ions that are capable of catalyzing reaction. (iii) Secondary antioxidants prevent oxidation by decomposing lipid peroxides into stable end products (Romero et.al, 2007).

Donating electrons or hydrogen atoms to terminate a free radical reaction is performed by free radical scavengers (Halliwell et al., 1995). The activity of phenolic compounds to scavenge the free radical is generally related with their ability to donate a hydrogen atom for decreasing ROS radicals (Halliwell et al., 1995). In this reaction, phenolic compounds are changed to oxidized phenoxy radicals (ArO·) that are stable due to resonance-stabilized delocalization of the unpaired electron over the aromatic ring (Pietta, 2000; Aruoma, 2002). For instance, peroxyl and hydroxyl radicals can be reduced by the phenolic compounds as in the following reaction:

$$ROO\cdot + ArOH \rightarrow ROOH + ArO\cdot$$

$$HO\cdot + ArOH \rightarrow HOH + ArO\cdot$$

where ArOH represents the phenolic compound and ArO· is the phenoxy radical.

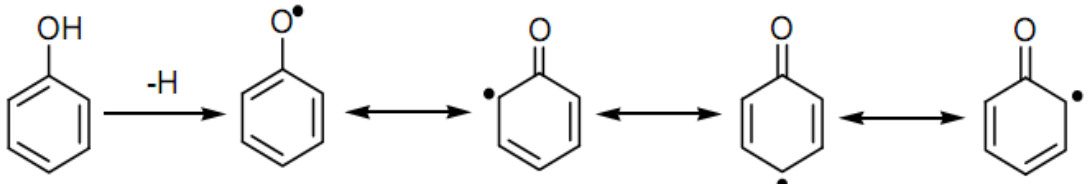


Figure 1.7. Delocalization of the unpaired electron on a phenol radical (Adapted from Gordon, 1990).

1.4.2. Potential Health Benefits of Phenolic Compounds

Regular consumption of foods rich in phenolic compounds (fruits, vegetables, whole grain cereals, red wine, tea) bringing about to a reduced incidence of cardiovascular diseases, neuro-degenerative diseases and certain forms of cancer have been shown by epidemiological studies (Hung et al., 2004; Lasheras et al., 2000; Halliwell, 1994). This beneficial effect is mainly ascribed to the presence of vitamins, minerals and secondary phytochemicals such as carotenoids, anthocyanins, flavonoids, and other phenolic compound distributed throughout the plant kingdom (Anttonen and Karjalainenb, 2005; Giuntini et al., 2005; Lasheras et al., 2000). The consumption of tomato has been advised for decreasing the incidence of various chronic diseases such as cardiovascular diseases and certain cancer forms particularly prostate cancer (Hollman et al., 1996; Rao et al., 1999). Furthermore, the consumption of tomato results in reduced levels of serum lipid and oxidation of low-density lipoprotein (Agarwal et al., 2001). These beneficial effects have been related with the presence of important of antioxidants such as lipid-soluble lycopene and β-carotene, soluble Vitamin C, and compounds of intermediate hydrophobicity such as quercetin gylcosides, naringenin chalcone, and chlorogenic acid in tomatoes (Abushita et al., 2000). Specifically, cherry consumption has been reported to alleviate arthritis and gout-related pain (Wang et al., 1999; Seeram and Nair, 2002).

It has been reported that the phenolic compound of green tea prevented intestinal uptake of glucose through rabbit intestinal epithelial cells, and this could cause a reduction of blood glucose levels. Epigallocatechin gallate, which is a phenolic compound of green tea, has been known to decrease the risk of tumours in the

esophagus, liver, lungs, skin and stomach of experimental animals (Huang et al., 1992; Shahidi and Naczk, 2004).

Yamakoshi et al. (2002) found that the formation of cataract can be prevented by the grape seed consumption in rats as the antioxidant activity of proanthocyanidin-rich extracts of grape seed can tolerate the oxidative stress causing cataract formation.

1.5. Phenolic Compound Extraction from Industrial By-Products

Hasbay et al. (2008) studied on the extraction of total phenolic compounds (TPC) from sour cherry pomace by high pressure liquid extraction (HPE) and supercritical fluid extraction (SFE). In addition, antiradical efficiency (AE) of the extracts was determined. For HPE, TPC, and AE at the optimum conditions (176-193 MPa, 60 °C, 0.06-0.07 g solid/ml solvent, 25 min) were found as 3.80 mg GAE/g sample and 22 mg $DPPH^{\cdot}$/g sample, respectively. On the other hand, TPC and AE at the optimum conditions (54.8-59 MPa, 50.6-54.4°C, 20 wt% ethanol, 40 min) for SFE were determined as 0.60 mg GAE/g sample and 2.30 mg $DPPH^{\cdot}$/g sample for sour cherry pomace, respectively.

Garcia et al. (2009) analyzed eleven different cider apple pomaces for phenolic profiles and antioxidant capacity. The Folin index varied from 2.3 to 15.1 g gallic acid per kg of dry matter. Flavanols, dihydrochalcones (phloridzin and phloretin-$2'$-xyloglucoside), flavonols and cinnamic acids (chlorogenic and caffeic acids) were major phenols detected in this study. The flavanol contents ranged between 1.7 and 2.5 g/kg. Phloridzin was always the main dihydrocalcone present in the apple pomaces, with contents ranging between 0.6 and 1.5 g/kg. Phloretin-$2'$-xyloglucoside was the second one, with contents in the range of 0.08 and 1.0 g/kg. Considering phenolic acids, their contents were between 0.5 and 1.6 g/kg, being chlorogenic acid the major one in all the cases. The antioxidant activities of cider apple pomaces varied from 4.1 to 14.5 (FRAP assay), or from 4.4 to 16.0 (DPPH assay) g ascorbic acid/kg DM. In the study of Wolfe and Liu (2003), apple peels were determined to

contain up to 3300 mg/100 g dry weight of phenolics, while the lypholisate obtained from apple pomace contained 118 mg/g of phenolics (Schieber et al., 2003).

Lee and Wrolstad (2004) studied the total antocyanins and total polyphenolics of blueberry processing waste. They reported that blueberry skins indicated the highest total antocyanin, total polyphenolics, and antioxidant activity compared with flesh and seed fractions.

In the study of Su and Silva (2006), the aim was to investigate the effects of fermentation type on retention of total anthocyanins, total phenolics, and antioxidant activity of blueberry by- products. Therefore, total phenolics (TPC), total anthocyanins, antioxidant activities (β-carotene bleaching assay and ferric thiocyanate assay), and antiradical activity (DPPH radical-scavenging assay) of rabbiteye blueberry by products (juice, wine, and vinegar pomaces) were analyzed. The results indicated that the wine pomace had the higher TPC, antioxidant activity and antiradical activity; in contrast, the vinegar pomace possessed the lowest anthocyanins, TPC, antioxidant activities and antiradical activity. It could be concluded that the acetification process during vinegar production resulted in a significant decrease in the TPC, anthocyanins, antiradical activity, and antioxidant activities, but an important portion of phenolics and antioxidant activity were still retained by the vinegar pomace.

Larrauri et al. (1996) investigated extractable polyphenols and antioxidant activities of white and red grape pomaces. Total extractable polyphenols in white grape pomace (6.5%), skins (5.3%) and seeds (9.2%) were higher than those in red grapes: 2.2%, 2.1%, and 2.1%, respectively. Red grape skin antioxidant activity determined by the ferric thiocyanate method was the strongest, but it was 1.5 to 2 times lower than that of red wine.

As the phenolic content depends on grape variety, and vinification, Thimothe et al. (2007) investigated the chemical composition and biological activity of phenolic extracts prepared from several red wine grape varieties and their fermented pomace

of winemaking on some of the virulence properties of *Streptococcus mutans*. When the grape was compared to the fermented pomace, the anthocyanins and flavan-3-ols content were highly different due to the grape variety and type of extract. The glycolytic pH-drop by *Streptococcus mutants* cells was inhibited by the pomace extract without influencing the bacterial viability. This study showed that the biological activity of fermented pomace was significantly better than whole fruit grape extracts.

The by-products of grape juice and white wine production, which are grape seeds and skin, are considered as a source of phenolic compounds, especially mono-, oligo-, and polymeric proanthocyanidins (Shrikhande, 2000; Torres and Bobet, 2001). Total extractable phenolics of fresh grape are distributed as 10% in pulp, 60-70% in seeds, and 28-35% in skin. The phenolic content of grape seeds varies from 5% to 8% by weight (Shi et al., 2003).

Kammerer et al. (2004) evaluated phenolic compounds of fourteen pomace samples originating from red and white winemaking by HPLC-MS. According to their result, large differences were observed in all individual phenolic compounds as related with the cultivar and vintage. Grape skins were determined as the part being rich in anthocyanins, hydroxycinnamic acids, flavanols, and flavonol glycosides, and flavanols were chiefly present in the seed part. Furthermore, although the white grape pomace suffers from the deficit of anthocyanins, no major difference was detected between red and white grape varieties.

The by-products of the olive industry, particularly olive mill wastes, have been considered to be a source of phenolic compounds. Ranalli et al. (2003) claimed that the annual production of olive mill wastes exceeds seven million tonnes. In addition, the total phenolic content of the olive mill waste water was observed to change between 1.0 % and 1.8% (Visioli and Galli, 2003) due to the varietal factors and processing effects. This waste water was found as rich with the phenolic components such as hydroxytyrosol, tyrosol, oleuropein, and a variety of hydroxycinnamic acids (Obied et al., 2005).

As the skin of fruits and vegetables are considered to be indigestible and containing low levels of nutrients, these parts are generally discarded during processing. However, it has been shown that the peels of several fruits are rich in antioxidants and have higher amounts of phenolic compounds than the flesh of fruits.

Apple polyphenolics are mainly present in the peel (Teuber and Herrman, 1978; Dick et al., 1987; Lommen et al., 2000) and in the seeds (Lu and Foo, 1998; Awad et al., 2000). However, most of the phenolics are kept in the pomace during juice production (Price et al., 1999).

The citrus industry produces large amounts of peels and seed residue, which may be considered up to 50% of the total fruit weight (Bocco et al., 1998). When the residue is utilized properly, it might be main sources of phenolic compounds. Gorinstein et al. (2001) observed that the total phenolic contents in peels of lemons, oranges, and grape fruit were higher than those of the peeled fruits.

Peels from apples, peaches and pears were reported to contain 50% higher amount of total phenolics than those of peeled fruits (Gorinstein et al., 2002). Similarly, Gil et al. (2002) found that peels of yellow and white flesh nectarines contained at least twice as much phenolics as the flesh.

Chang et al. (2000) investigated eight selected clingstone peach cultivars, and indicated that the peels exhibited 2-2.5 times the amount of total phenolics found in the edible flesh. On the other hand, Someya et al. (2002) observed that the edible pulp of banana contains 232 mg /100 g dry weight phenolics whose amount is four times higher than that of its peel. Likewise, pomegranate peel was compared with the pulp, and it was found that pomegranate peels contain 249.4 mg/g phenolics as approximately 10 times of the phenolic content (24.4 mg/g phenolics) of the pulp (Li et al., 2005).

1.6. Extraction of Phenolic Compounds

1.6.1. Soxhlet Extraction

Soxhlet extraction is one of the most common methods used in the isolation of phenolic compounds from solid samples. In conventional Soxhlet, the sample is placed in a thimble-holder, and filled with condensated fresh solvent from a distillation flask through the operation. With reaching of the fluid to the overflow level, a siphon aspirates the solute of the thimble-holder, and discharges it back onto the distillation flask. This operation is repeated until all extracted analytes are carried into the bulk fluid.

The most prominent advantages of conventional Soxhlet are as follows: enhancing sample - fresh solvent contact, requiring little specialized training to operate, and presenting a potential to extract more sample mass than most of the latest methods (microwave extraction, supercritical fluid extraction, etc). However, in comparison to other extraction techniques, it has longer extraction time, and thus may cause destruction of target compounds due to local overheating effects, and requires large amount of solvent that is being associated with the additional cost and environmental problems (Proestos and Komaitis, 2008). In addition, the conventional Soxhlet device suffers from the lack of the agitation which could accelerate the extraction process.

Most commonly used solvents are ethanol, acetone, ethyl acetate, diethyl ether, acetonitrile, 2-propanol and their mixtures in the extraction of phenolic compounds (Gil et al., 2002; George et al., 2004; Kanner et al., 1994; Karadeniz et al., 2005; Su and Silva, 2006).

1.6.2. Microwave Assisted Extraction (MAE)

In the last decade, the investigation and the application of the microwave energy has been increased in the extraction of analytes from matrices. Microwave energy results in molecular motion by migration of ions and rotation of dipoles. Microwave energy does not cause any changes in the molecular structures. As the microwave energy

does not have any effect on nonpolar solvents, such as hexane and toluene, the usage of polar additives is needed in microwave-assisted extraction (Santana et al., 2009).

Microwave-assisted extraction (MAE) has been thought as alternative to conventional techniques due to its several advantages such as shorter extraction time, less amount of solvent usage and higher extraction rate.

Microwaves are non-ionizing electromagnetic waves, whose frequency is between 300 MHz to 30 GHz, placed between the X-ray and infrared rays in the electromagnetic spectrum (Letellier and Budzinski, 1999). Microwaves are composed of two oscillating perpendicular field's, electric field and magnetic field being responsible for heating. The heating mechanism is different from conventional heating, in that, heating occurs in a targeted and selective manner. The extraction time are reduced significantly in MAE as compared to Soxhlet due to the heating mechanism (Huie, 2002). The heating principle by microwaves is related to the interaction of microwaves with polar materials, and is been controlled by two phenomenon called as ionic conduction and dipole rotation (Letellier et al., 1999; Letellier and Budzinski, 1999). In this heating mechanism, the microwave energy is transferred to the material effectively through molecular interaction with the electromagnetic field and provides a fast transfer of energy to the extraction solvent and raw plant materials (Criado et al., 2004; Zhou and Liu, 2006). The efficiency of heating of different solvents under microwave is dependent on the dissipation factor (tanδ), which represents the measure of the ability of the solvent to absorb microwave energy and pass it on as heat to the surrounding molecules (Zuloaga et al., 1999). The dissipation factor is given by the following equation:

$$\tan \delta = \varepsilon'' / \varepsilon', \qquad (1.1)$$

where ε'' is the dielectric loss factor which shows the efficiency of converting microwave energy into heat and ε' is the dielectric constant which indicates the measure of the ability to absorb microwave energy.

Table 1.2 shows the dielectric constants and dissipation factors for solvents widely used in the MAE. As can be seen in the table, since ethanol and methanol possess lower ε', it can be assumed that they will absorb lesser amount of microwave energy than water. However, the higher overall heating efficiency for both solvents is obtained than water due to high tan δ value. On the other hand, hexane and other less polar solvents like chloroform will remain transparent to microwave, and therefore no heat is produced during the process (Santana et al., 2009).

Table 1.2. Dissipation factor and dielectric constants for some solvents commonly used in MAE (Adapted from Mandal et al., 2007).

Solvent	**Dielectric constant[a] (ε')**	**Tanδ x10^4**
Acetone	20.7	5555
Ethanol	24.3	2500
Hexane	1.89	1.01
Methanol	32.6	6400
2-propanol	19.9	6700
Water	78.3	1570

a: determined at 20°C

In plant material, water acts as the target for microwave heating, in that, the moisture inside of plant cell is heated due to the microwave effect, evaporates and produces a great pressure on the cell wall (Wang and Weller, 2006). The pressure pushes the cell wall from inside, stretching and rupturing it, and this enhances the leaching out of the active constituents from ruptured cells to the surrounding solvent, and therefore the

yield of phyto-constituents can be increased. When the plant matrix is exposed to the solvents with high dissipation factor under microwave, the possibility of obtaining the target compounds can be more intensified.

The effect of microwave is strongly related with the dielectric susceptibility of both solvent and solid plant matrix. In most cases, the sample is mixed with a single solvent as well as the mixture of solvents absorbing microwave energy strongly (Zuloaga et al., 1999).

Two kinds of MAE systems are used commercially: either with closed vessels (under controlled pressure and temperature), or with open vessels (under atmospheric pressure).

Hayat et al., (2009) investigated the optimization of microwave-assisted extraction of phenolic acids from citrus mandarin peels with the comparison of ultrasound and rotary extraction. Extraction using 66% methanol as a solvent and solvent to solid ratio of 16 at 152 W microwave power for 49 s was found to be the optimum condition. MAE was found to be a better method than other methods due to its being easy and rapidness to achieve higher yields and higher antioxidant activity. It was concluded that MAE could be suitable to be replaced with the traditional time-consuming methods in the extraction of phenolic compounds from citrus mandarin peels.

Xiao et al. (2008) studied on the fast extraction of flavonoids from *Radix Astragali* using MAE. The optimum yield of flavanoids with MAE was found as 1.190 ± 0.042 mg/g which was close to that of Soxhlet extraction (1.292 ± 0.033 mg/g), and higher than that of ultrasound assisted extraction (0.736 ± 0.038) and heat reflux extraction with 90% ethanol (0.934 ± 0.021). It was concluded that MAE reduced the extraction time and accomplished higher amount of flavanoids with no degradation, and therefore this gives a possibility of using this approach for industrial application in the coming future.

In the study of Rostagno et al. (2007), MAE of soy isoflavones was optimized at 500 W, and the optimum extraction conditions were found as 0.5 g of sample, 50 °C, 20 min and 50% ethanol as extraction solvent without degradation of isoflavones.

Beejmohun et al. (2007) performed MAE for the main phenolic compounds in flaxseed. It was found that higher yields were obtained by MAE as compared to traditional extraction. Furthermore, the former method required less extraction time and fewer steps, compared with conventional extraction methods.

MAE was also used in the extraction of tea polyphenols and tea caffeine from green tea leaves by Pan et al. (2003). As a result, the extraction of tea polyphenols and tea caffeine with MAE for 4 min was found to be higher than those of extraction at room temperature for 20 h, ultrasonic extraction for 90 min and heat reflux extraction for 45 min. MAE was more efficient, compared to the traditional extraction methods with respect to extraction efficiency and the percentages of tea polyphenols or tea caffeine in extracts. As being similar with other studies, MAE procedure offered high extraction, high extraction selectivity, shorter time and less labor intensive.

Duverney et al. (2005) used MAE in the extraction of vitamin E from rice bran. MAE was found to result in higher yields of tocopherols and tocotrienols from rice as compared to other methods.

Sterbova et al. (2004) combined microwave-assisted isolation and solid phase purification procedures before the chromatographic determination of phenolic compounds in plant materials. Combination of MAE with solid phase extraction offers a practical technique for isolation and purification of target compounds. The main advantages of MAE procedure were having highly repeatable results and high efficiency of the SPE clean-up step.

Although various food sources have been investigated to determine total phenolics by using several extraction methods, there is no study in literature on microwave-assisted extraction of tomato and sour cherry phenolic compounds.

1.6.3. Solid Phase Extraction

The differential migration process, in which analytes are absorbed in a solid sorbent, and then eluted by elution solvent, is the basis of solid-phase extraction method (SPE). The used devices of SPE are cartridges, columns and syringes (Santana et al., 2009). Various organic solvents such as ethyl acetate, methanol, acetonitrile or acetone are used to elute retained analytes into sorbents (Bagheri et al., 2004; Saitoh et al., 2002). The chosen solvent type varies according to the kind of sorbent and the polarity of each analyte. Although SPE method can be performed in the off-line and on-line modes, on-line use of SPE is more common due to the advantages such as higher sensitivity and less manipulation of the samples (Bagheri et al., 2004; Saitoh et al., 2002). SPE-HPLC method provides easy and simultaneous determination of flavonoids and other phenolic compounds in fruit juices (Chen et al., 2001; Picinelli et al., 1997).

Palma et al. (2002) used a new method of pressurized-fluid extraction coupled in-line with SPE performed under an inert atmosphere using five different extracting solvents as water, ethanol, diethyl ether, ethyl acetate and methanol for the determination of phenolic compounds. In this study, the in-line coupling of pressurized fluid extraction (PFE) and SPE provided the successful extraction due to the SPE's ability of decreasing the sample handling, and occurring the lower risk of degradation of the extracted compounds. It was also concluded that extraction methods with enhanced selectivity would be developed when similar methods are applied to other samples.

Similarly, Papagiannopoulos et al., 2002 performed a new instrumental setup combining pressurized liquid extraction coupled on-line with SPE for the automated extraction of proanthocyanidins in malt, and used HPLC for the quantitative determination of this compound. This application was found as to reduce analysis time and to improve the recovery.

Fiorentino et al. (2006) compared the traditional liquid-liquid separation into phenolic and aliphatic fractions with modern and versatile fractionation technique using SPE on aminopropylbonded phases. The similar conclusion as obtained in mentioned previous studies was reached that the SPE method enables not only a larger recovery of compounds with reduced sample and solvent amount, but also larger ease and rapidity of sample handling than the traditional liquid-liquid separation. As a result, this method could be recommended in structural studies of natural organic matter.

For the concentration of isoflavones, SPE was used by Rostagno et al. (2005). The method succeeded high isoflavone recoveries (more than 98%) with being fast (less than 10 min), highly accurate and reproducible. This method gave a chance to concentrate the sample to allowing the measurement of isoflavones at low concentrations.

1.6.4. Ultrasound Assisted Extraction

The ultrasound assisted extraction (UAE) can be used for the extraction of analytes in solid matrixes with the application of liquid solvents. As this method is compared with the traditional methods, it can be considered as faster due to the large contact area between solid and liquid phase and occurring better particle scattering (Filgueiras et al., 2000). The advantages of this method over other extraction techniques may be intensification of mass transfer, cell disruption, enhanced penetration and capillary effects. The increase in the mass transfer results from the occurrence of very high temperatures that could enhance the solubility and diffusivity, and pressures, which favor penetration and transport (Luque-Garcia and Luque de Castro, 2003). However, it possesses two shortcomings which are the lack of uniformity in the distribution of ultrasound energy and the decrease of power with time.

Virot et al. (2009) investigated the extraction efficiency in terms of time needed and total polyphenol content of apple pomace using UAE and conventional extraction.

The yield was increased more than 20% when UAE was used and extraction time was reduced as compared to conventional extraction.

In another study, Fu et al. (2006) used UAE for the isolation of xyloglucan from apple pomace. It was found that the ultrasound assisted extraction of xyloglucan was about three times faster than the traditional extraction method.

The phenolic compounds from pistachio hull were extracted using three different solvents, water, methanol and ethyl acetate by two different extraction methods, solvent extraction and UAE (Goli et al., 2005). They concluded that no significant difference was observed in the extraction yields between the solvent extraction and UAE.

UAE was compared with solid-liquid, subcritical water extraction and microwave assisted extraction in the determination of phenolic compounds of strawberries (Herrera and Luque de Castro, 2005). According to the results, it was shown that UAE was found to be much faster and degrading less target compounds than solid-liquid, subcritical water and microwave-assisted extraction methods.

1.6.5. Supercritical Fluid Extraction

In the supercritical fluid extraction (SFE), the sample is placed into an extraction vessel, which is combined with the temperature controllers and pressure valves at both inlet and outlet to maintain the desired extraction conditions. This process is performed in two steps. In the first step the target compounds are made soluble by the supercritical fluid and extracted from the matrix. In the last step, the vent valve is opened and the soluble compounds are retained in a liquid solvent commonly in ethanol or methanol as well as in an inert solid matrix. The main supercritical solvent is carbondioxide. Carbondioxide is cheap, environmentally friendly and generally identified as safe by FDA. Supercritical CO_2 is attractive because of its high diffusivity combined with its solvent strength. Another advantage is that CO_2 is gaseous at room temperature and pressure, which makes analyte recovery very simple and provides solvent-free analytes.

Grigonis et al. (2005) compared SFE, MAE and Soxhlet extraction for the isolation of antioxidants from sweet grass. Among these extraction methods, the highest extraction yield was found in Soxhlet extraction (0.58%), followed by SFE (0.46%), Soxhlet + microwave-assisted extraction (0.38%) and microwave-assisted extraction (0.30%). As a result, this study indicated that both MAE and SFE extractions might be used to get antioxidants from sweet grass.

Palma and Taylor (1999) performed near critical CO_2 for the extraction of phenolic compounds from white grape seeds. In performing this operation, several parameters such as CO_2 density, organic modifier, percentage of modifier and extraction temperature were optimized. Under optimized conditions, SFE using methanol-modified CO_2 yielded higher catechin and other phenolic compound recoveries from grape seed than liquid-solid extraction. Although SFE was found as being less reproducible, it has been still advised due to its being fast and lower incidence of degradation reactions.

Hasbay et al. (2008) extracted total phenolics (TPC) of sour cherry pomace by high pressure liquid extraction (HPE) and subcritical fluid (CO_2 + ethanol) extraction (SCE) and also determined antiradical efficiency (AE) of these extracts. According to their results, for HPE, TPC and AE at the optimum conditions were determined as 3.80 mg GAE/g sample and 22 mg DPPH˙/g sample, respectively. On the other hand, TPC and AE were significantly lower in the extracts obtained at optimum conditions of SCE and they were found as 0.60 mg GAE/g sample and 2.30 mg DPPH˙/g sample, respectively.

1.6.6. Pressurized Liquid Extraction (PLE)

Pressurized liquid extraction is a technique which combines elevated temperature and pressure with liquid solvents to achieve fast and efficient extraction of the analytes from the solid matrix. The higher temperatures results in a decrease in solvent viscosity and thereby increasing the solvent's ability to wet the matrix and to solubilize the target analytes. In addition, the temperature improves the breakdown

analyte-matrix bonds and encourages analyte diffusion to the matrix surface (Carabias-Martínez et al., 2005).

Ju and Howard (2003) used PLE to extract anthocyanins from the freeze-dried skin of a highly pigmented red wine grape with six solvents (0.1% HCl in deionized water (pH 2.3); 0.1% HCl in 60% ethanol (pH 2.2); 0.1% HCl in 60% methanol (pH 2.3); 0.1% HCl in 40:40:20 (methanol/acetone/water) (pH 1.9); 7% acetic acid in 70% methanol (pH 2.0) and 0.1% trifluoroacetic acid in 70% methanol (pH 2.1) at 50 °C, 10.1 MPa, and 3 × 5 min extraction cycles. Acidified methanol extracted the highest level of total monoglucosides and total anthocyanins, but the solvent mixture extracted the highest amount of total phenolics and total acylated anthocyanins. Optimum temperatures for the extraction of total anthocyanins were determined as 80-100 °C for acidified water, and 60°C for acidified 60% methanol. In conclusion, the researchers showed that high-temperature PLE using acidified water is effective for extracting anthocyanins from grape skins.

Alonso-Salces et al. (2001) extracted phenolic compounds from Golden Delicious apple peel and pulp by PLE. The influence of solvent composition (0-100% methanol in water), temperature (40-100°C), static extraction time (5-15 min) and pressure (6.9-10.3 MPa) on the extraction efficiency were investigated. According to the results, the optimum extraction conditions were determined as pure methanol, 40°C, 5 min and 6.9 MPa. As a result, the efficiency of PLE was found to be comparable to conventional techniques in the extraction of phenolic compounds from apple peel and pulp.

1.7. Objectives of the Study

The investigation of phenolic compounds in plants has attracted significant attention because of the claims related to their therapeutic or health improving properties. Phenolic compounds are antioxidant substances and these compounds postpone or prevent the oxidation of an oxidisable substrate even though antioxidant substances are present in foods at lower concentrations than oxidisable substrate (Laroze and Zuniga-Hansen, 2007). In food industry, synthetic antioxidants such as butylated hydroxyanisole, butylated hyroxytoluene, propyl gallates and tert-butylhydroquinone are commonly preferred due to their high effectiveness and low cost. However, the toxicity of these compounds and the demand of consumers to consume the food with no additives have started the necessity to identify natural and safe sources for food antioxidants. In addition, high amount of the pomaces arising from the processing of fruits and vegetables are usually utilized as feed or fertilizer or left as industrial waste. Nevertheless, the pomaces are good raw materials for achieving extracts rich in phenolic compounds with favorable antioxidant properties.

Since conventional techniques for the extraction of phenolic compounds is time consuming and thermally unsafe, there is a recent research interest in the extraction of phenolic compounds by using microwave assisted extraction technique (MAE). As no studies have been presented on MAE of tomato and sour cherry pomace in the literature, it was aimed to optimize the extraction conditions of tomato and sour cherry pomaces.

The objective of this study was to compare MAE and conventional extraction techniques for the extraction of phenolic compounds from sour cherry and tomato pomaces. Antiradical efficiency (AE) of the extracts and concentration of phenolic compounds in the extracts were also determined. Moreover, the effects of microwave power, solvent type, extraction time and solvent to solid ratio on phenolic compounds of sour cherry and tomato pomace were studied.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

The residue part, which is obtained after processing of fruits in the juice, wine or other products' production, is called as pomace. The pressed skins, pulp residue, seeds and stems are included in the pomace and they are rich in phenolic compounds (Su and Silva, 2006). Sour cherry (*Prunus cerasus*) and tomato (*Lycopersicon esculentum Mill*) pomaces were taken from the fruit juice production pilot plant of Ankara University, Department of Food Engineering. The pomace was mixed well and placed in 20×25 cm plastic bags and stored at -80°C.

The pomace was freeze-dried at -53 °C and 0.02 kPa (Christ Alpha 1-2 LD Plus, Germany) for 24 hours. The moisture content of the freeze-dried pomaces was determined by putting about 2 g of pomace in previously dried and weighed containers and keeping it at 100°C until constant weight was reached. The freeze-dried samples were ground using kitchen-type grinder (Premier, South Korea) The sauter mean diameter of samples was calculated as 0.520 mm by sieve analysis. The dried and ground samples were kept at -30°C.

Folin-Ciocalteau's phenol reagent (SIGMA F9252), sodium carbonate (SIGMA S7795), 1,1-Diphenyl-2-picrylhydrazyl (SIGMA D9132), absolute ethanol (SIGMA 32221) and gallic acid (SIGMA 7384) were used as reagents for performing the analyses. In addition, vannilic acid, (-) epicatechin, syringic acid, gentisic acid and quercitrin were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Microwave Assisted Extraction (MAE)

The microwave assisted extraction was performed using Ethos D microwave system (Milestone, Italy), which operates at a maximum power of 1000 W. The extractor was modified by replacing its vessels with a boiling flask of 250 mL capacity and a condenser. Microwave-assisted extraction apparatus can be seen in Figure 2.1.

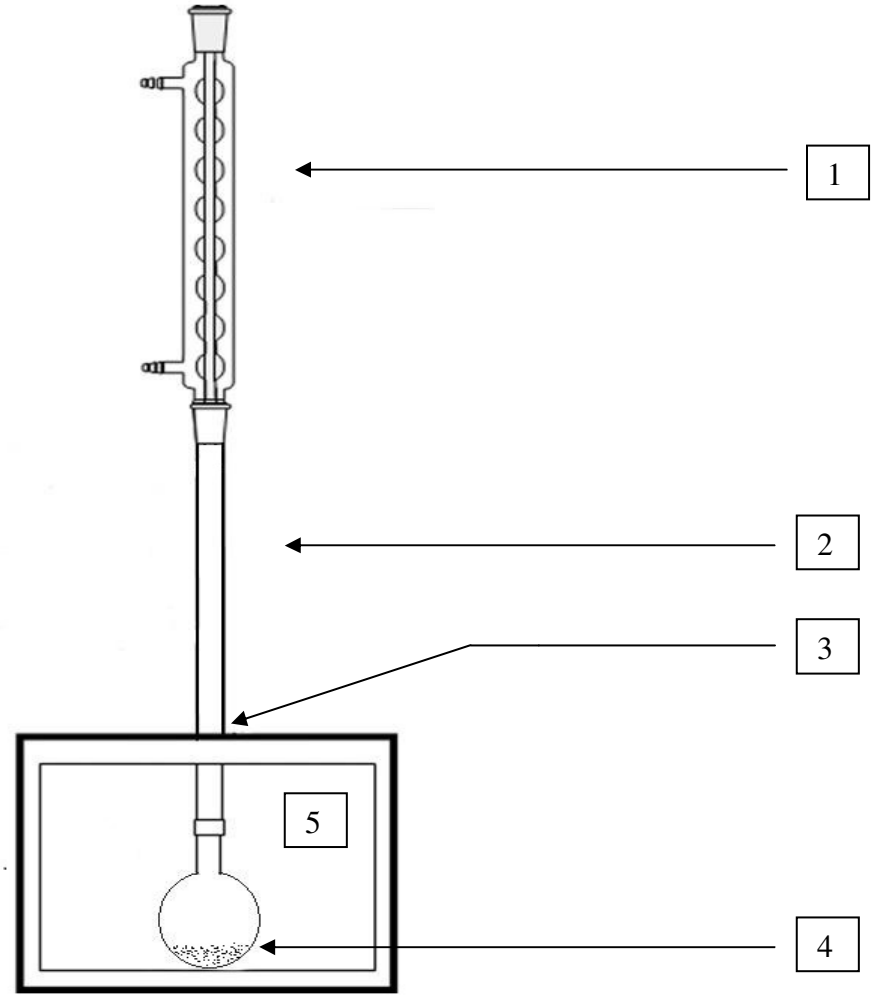


Figure 2.1. The microwave-assisted extraction system. 1.condenser, 2. adapter, 3.opening on upper surface of the microwave oven, 4.solvent-solid mixture, 5. Microwave oven.

Four experimental parameters (solvent type, solvent to solid ratio, extraction time and microwave power) were chosen in the study. One gram of freeze-dried sample was extracted with three different solvents which are ethanol, water and ethanol-water mixture (1:1 v/v) at different solvent to solid ratios (10, 20 and 30 ml/g) under two different microwave power levels (400 and 700 W) for four different extraction times. Power was determined by IMPI-2 L test. Extraction time intervals were determined by performing preliminary experiments and they differed according to the power and sample. Extraction of tomato pomace was performed at 400 W for 12, 14, 16, 18 and 20 min and at 700 W for 10, 12, 14 and 16 min. For the extraction of sour cherry pomace at 400 W, extraction times were 10, 12, 14 and 16 min. When the power was increased to 700 W, extraction was performed for 8, 10, 12 and 14 min. After completing the extraction process, the extract was filtered (0.45 µm) and stored in brown colored glass bottles of 50 ml capacity at + 4°C in the refrigerator.

The maximum power of the microwave oven was determined using International Microwave Power Institute (IMPI) 2L test (Buffler, 1993). First, the oven was heated by operating at 100% power for 5 min with a load of 2000 ± 5 g water placed in two 1-L Pyrex beakers. The oven cavity was wiped with a wet cloth. The subsequent procedure was as follows: Two beakers of 1-L capacity, each containing 1000 g of water, were placed in the center of the oven, side by side and touching each other in the width dimension of the cavity. Initial temperature of the water in the beakers was 20°C ± 2°C. The oven was operated for 2 min 2 s at 100% power level. Final temperatures were measured immediately after the oven was turned off. Three replications were made and the oven cavity was wiped with a wet cloth each time. The power was calculated using the following formula:

$$P(W) = mCp\frac{\Delta T}{\Delta t} \qquad (2.1)$$

where ΔT is the temperature rise of the water in the two beakers calculated by subtracting the initial water temperature from final temperature.

2.3. Conventional Extraction

Tomato and sour cherry pomace were extracted using three different solvents for 6 hours. This extraction time was determined by the preliminary experiments. The solvent to solid ratio and solvent types were the same as in MAE experiments. The sample was placed into the flask by containing extraction solvent. The only difference between conventional and MAE apparatus was the usage of hot plate instead of microwave for heating in conventional extraction.

2.4. Analysis of Extracts

The extracts of tomato and sour cherry pomace were analyzed to determine their total phenolic content, antioxidant activity and concentrations of their phenolic compounds.

2.4.1. Determination of Total Phenolic Content (TPC)

Total phenolic content was estimated by the Folin-Ciocalteau assay (Sigleton and Rossi, 1965). Change in color detected at 765 nm as a result of the reduction of the Folin-Ciocalteau reagent by phenolates generated in the presence of sodium carbonate is the basis of this method.

First of all, the gallic acid and the sodium carbonate stock solutions were prepared. For the gallic acid stock solution, in a 100 ml volumetric flask, 0.5 g of dry gallic acid was dissolved in about 10 mL of the used solvent and diluted to 100 mL with deionised water, ethanol or water-ethanol (1:1 v/v). For the sodium carbonate solution, 7.5 grams of sodium carbonate was dissolved in 70 mL of deionised water while being heated on the magnetic stirrer until boiling. After that, the solution was kept for 24 hours. Then, the solution was filtered, and deionised water was added to bring the total volume to 100 ml. This would make the solution concentration as 75 g/mL.

Then, 0.1 mL of extract was taken into a test tube and 0.9 mL of deionised water was added. After vortex mixing, 1.25 mL of 0.2 N Folin-Ciocalteau's phenol reagent was

added and the mixture was kept for 5 min at room conditions. At the end of 5 min, 1 mL of 75 g/L sodium carbonate solution was added into the tube, and the vortex mixing was applied to the mixture and incubated in dark at room temperature for 2 hours. The absorbance values were measured at 765 nm using spectrophotometer (S-22 Boeco UV Visible Spectrophotometer, Hamburg, Germany). The spectrophotometric measurement was repeated three times for each extract and the average value was used to determine total phenolic content from gallic acid calibration curve (Figure A1-A3). The result was expressed as mg of gallic acid/g of dry sample. The standard curves were prepared using gallic acid solutions at different concentrations prepared with solvent types used in extraction.

2.4.2. Determination of Antioxidant Activity

Antioxidant activity of the extracts was determined by detecting the scavenging of DPPH$^{\cdot}$ (1,1-diphenyl-2-picrylhydrazyl) radical. For the determination of plateau, different concentrations of gallic acid was prepared in ethanol, and 0.1 ml of gallic acid was mixed with 3.9 ml of 0.025 mg/ml DPPH$^{\cdot}$ in a tube in the dark place at room temperature for 2 h. The absorbance values were measured at different time intervals ranging from t=0 until the absorbance values reached to a plateau, and therefore the holding time of the samples was determined as 60 min. For the analysis, 0.1 ml of the extract was placed in tubes and 3.9 ml of 0.025 mg/ml DPPH$^{\cdot}$ was added to the extracts. After the tubes were held in the dark place at room temperature for 60 min, the absorbance values were read using ethanol as blank at 515 nm (S-22 Boeco UV visible spectrophotometer, Hamburg, Germany), and then converted to DPPH$^{\cdot}$ concentration using the standard curve. The equation for the standard curve was:

$$A_{515} = 0.2335\,[DPPH^{\cdot}] + 0.3122 \qquad (R^2=0.998) \qquad (2.2)$$

where A_{515} is the absorbance at 515 nm and remaining DPPH$^{\cdot}$ concentration can be calculated from;

$$\%\ DPPH^{\cdot}_{rem} = ([DPPH^{\cdot}]_t / [DPPH^{\cdot}]_{t=0}) \times 100 \qquad (2.3)$$

where $DPPH^{\cdot}_{rem}$ is the remaining concentration of $DPPH^{\cdot}$, $[DPPH^{\cdot}]_t$ is $DPPH^{\cdot}$ concentration read at definite time (t), and $[DPPH^{\cdot}]_{t=0}$ express initial $DPPH^{\cdot}$ concentration.

The percentage of remaining $DPPH^{\cdot}$ against the standard concentration was then plotted and presented in Figure A4 to obtain EC_{50} that can be described as the amount of antioxidant needed to reduce the initial $DPPH^{\cdot}$ concentration by 50%. An example for the determination of EC_{50} for gallic acid is introduced in Figure A5. Finally, antioxidant activity was stated in terms of antiradical efficiency (AE) which is the inverse of EC_{50} (Monsouri et al., 2005).

2.4.3. Determination of Phenolic Acids by HPLC

Varian ProStar HPLC system (California, USA) consisting of Varian Prostar 410 Autosampler, Varian 330 PDA detector, and data processor of a Millenium 32 was used for the HPLC analysis. Pursuit C18 column (5 μm, inner diameter 4.6 m length) and MetaGuard Pursuit column (5 μm, 4.6 mm) were employed for the analysis of phenolic acids. The analysis was performed using a gradient program with a two solvent system (A: formic acid (2.5% in water); B: methanol. Initial condition was 0% A; 0–42 min, changed to 80% A; 42–67 min, to 80% A; 67–68 min, to 100% A; 68–73 min to 100% A. The flow rate was always 1 mL/ min, and the injection volume was 50 μL. The signals were detected at 280, 300, 320, 340 and 360 nm for vannilic acid, epicatechin and syringic acid, gentisic acid and quercetin, respectively. Standard phenolic acids were prepared in solvent used in the extractions.

2.5. Statistical Analysis

Four way analysis of variance (ANOVA) was performed to determine whether the effects of microwave power, solvent type, extraction time and solvent to solid ratio on total phenolic content and antioxidant activity were statistically significant or not ($p \leq 0.05$). In addition, two extraction methods were compared. If significant difference was found, Tukey test was used for comparison. Throughout the statistical analysis, SPSS 16.0 (Chicago, United States) was used.

CHAPTER 3

RESULTS AND DISCUSSIONS

In the extraction of antioxidant compounds from plant materials, the selection of optimum conditions is critical. Since different natural antioxidants exist in different plant materials, the extraction conditions cannot be generalized (Wettasinghe and Shahidi, 1999). The influence of extraction parameters on the recovery of phenolic compounds from by-products of tomato and sour cherry juice processing has not been reported yet.

3.1. Extraction of Phenolic Compounds from Tomato Pomace

In this study, the effects of different microwave power levels (400 W and 700 W), solvent types (water, ethanol and ethanol-water mixture), extraction times (10, 12, 14, 16 and 18 min) and solvent to solid ratios (10, 20 and 30 ml/g) on TPC, AE and concentration of major phenolic compounds obtained by MAE were studied. In addition, MAE was compared with conventional extraction. Experimental data are shown in Appendix B (Table B.1 and B.2). Moreover, moisture content of tomato pomace was determined as 12.25%.

3.1.1. Total Phenolic Content of Tomato Pomace Extracts

Figure 3.1, 3.2 and 3.3 show the change of TPC of tomato pomace extracts obtained for different extraction times, solvent types and solvent to solid ratios when the power was kept constant at 400 W. As can be seen in these Figures, TPC values increased with increase in microwave extraction time, reached to maximum at 16 min, and then decreased, when ethanol-water and ethanol were used as extraction

solvents. When water was used as a solvent, the optimum extraction time was prolonged for 2 min, that is, the highest TPC was obtained at 18 min. Time was found to be a significant ($p \leq 0.05$) parameter on TPC of tomato pomace extracts Appendix C, Table C.2). The representation of experimental parameters used in statistical analysis was shown in Table C1. The decrease in TPC after the optimum extraction time might be explained by the overexposure of sample to microwaves. This might lead to the destruction of some phenolic compounds. Xiao et al. (2008) also showed that there was an optimum extraction time for the extraction of flavonoids from *Radix Astragali.* In addition, Pan et al. (2003) found that the amount of total polyphenols increased with the increase in time until 4 min, and then remained constant.

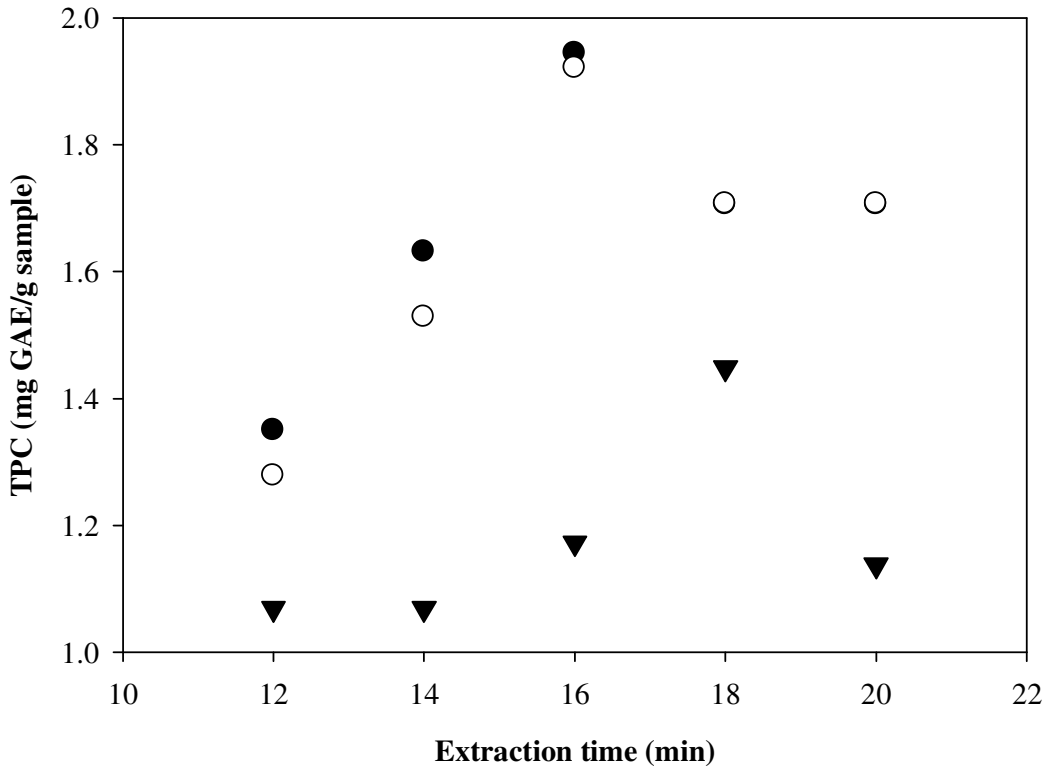


Figure 3.1. Effects of different solvent types on TPC of tomato pomace extracts obtained using solvent to solid ratio of 10 ml/g during MAE at 400 W. (●): ethanol-water, (○): ethanol, (▼): water.

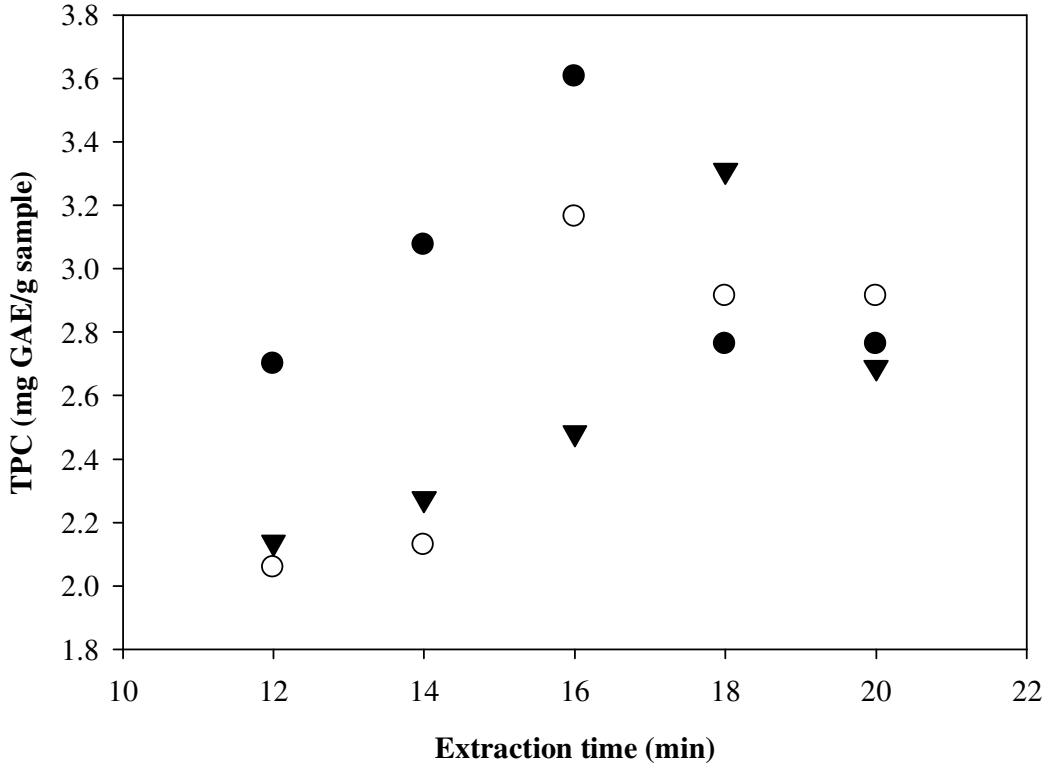


Figure 3.2. Effects of different solvent types on TPC of tomato pomace extracts obtained using solvent to solid ratio of 20 ml/g during MAE at 400 W. (●): ethanol-water, (○): ethanol, (▼): water.

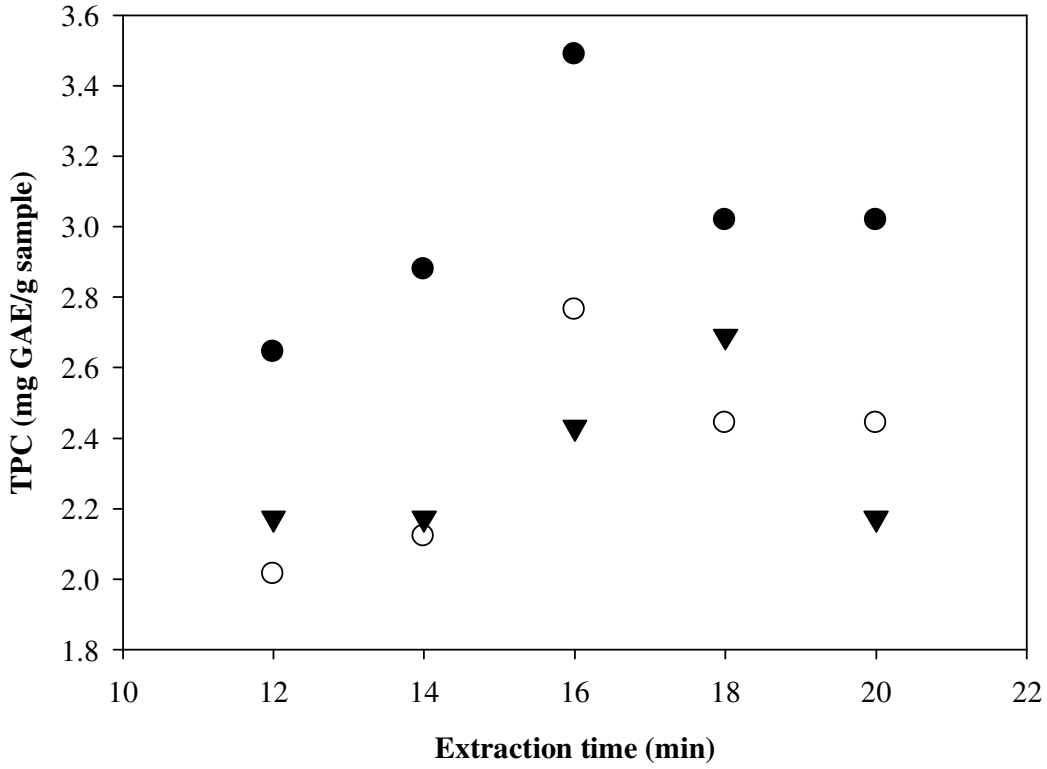


Figure 3.3. Effects of different solvent types on TPC of tomato pomace extracts obtained using solvent to solid ratio of 30 ml/g during MAE at 400 W. (●): ethanol-water, (○): ethanol, (▼): water.

When the effects of solvent types on TPC of tomato pomace extracts were compared, it was seen that the highest TPC was obtained in the case of ethanol-water mixture (Figure 3.1-3.3). Usage of ethanol-water mixture for extraction provided higher TPC as compared to other solvents as seen in Appendix C (Table C.2). The difference in the effectiveness of extraction solvents in MAE is related to the dissipation factor (tanδ) (Mandal et al., 2007) which is defined as the ratio of dielectric loss factor to dielectric constant. Therefore, choosing a solvent possessing a high dielectric constant as well as a high dissipation factor is very important in MAE to facilitate heat distribution through the matrix (Proestos and Komaitis, 2008). Generally, ethanol, methanol, water, ethanol-water, methanol-water, and acetone can be used for

MAE (Chen et al., 2008; Proestos and Kamaitis, 2008, Xiao et al, 2008; Hayat et al., 2009, Rostagno et al., 2007). Although methanol has higher dissipation factor than ethanol and water (Proestos and Komaitis, 2008), in this study it was not preferred as an extraction solvent since it is highly toxic and not safe for food processing. Since water has significantly lower dissipation factor (tanδ) than other solvents as represented in Table 1.2., lower amounts of extractable phenolic compounds were obtained. When water was used as a solvent, "superheating" can occur, and the possibility of degradation of the analyte may be increased due to higher rate of microwave absorption as compared to the rate of heat dissipation in the system (Proestos and Kamaitis, 2008).

Similar to our study, Pan et al., (2003) found that the ethanol-water mixture gave the highest amount of polyphenols in green tea as compared to 100% ethanol. The best ethanol to water ratio for the extraction of isoflavones using microwaves was found to be 50 (Rostagno et al., 2007). In another study, higher yield was obtained by using 60% aqueous ethanol as compared to extraction with 100% ethanol in the extraction of flavonoids from *Herba Epimedii* (Chen et al., 2008). Xiao et al. (2008) showed that the yield of the flavonoids from *Radix Astragali* increased with increase in ethanol concentration in water varying from 60% to 90%, and then decreased. All of the mentioned studies revealed that the extraction efficiency increased in the presence of some water. One possible reason leading this increase can be related to the increase in swelling of plant material by water and expanding the contact surface area between the plant matrix and the solvent (Chen et al., 2008 and Xiao et al., 2008). Furthermore, using water in combination with other organic solvents plays a significant role in the formation of a moderately polar medium that favors the extraction of phenolic compounds (Lapornik and Prosek; 2005 and Pathirana and Shahidi, 2005).

In MAE, the significance of dielectric properties should be considered in explaining the highest TPC values obtained in the presence of ethanol-water mixture. The

dielectric properties of ethanol-water mixture are higher than pure ethanol and water (Mudgett, 1995). The synergistic effect of ethanol-water mixture on dielectric properties is related to the chemical interaction, which stabilizes liquid structure by hydrogen bonding between ethanol and water molecules. For this reason, ethanol-water mixture is expected to be heated faster in MAE which results in better extraction yield.

The recovery of phenolic compounds using 400 W power level and optimum extraction times as a function of solvent to solid ratio can be seen in Figure 3.4. The optimum extraction times were determined from Figure 3.1-3.3 were used. It can be seen that TPC increased with the increase in solvent volume up to solvent to solid ratio of 20 ml/g and then decreased. These results were in agreement with most of MAE studies, in which higher solvent to solid ratio can lead to lower recoveries. Xiao et al. (2008) also reported that yield of flavonoids increased with the increase in solvent to material ratio and then decreased. In the presence of higher solvent volumes, heating may be restricted to the penetration depth of sample-solvent mixture. This may result in inefficient heating and as a consequence lower extraction yield as compared to lower solvent to solid ratios. In many studies performed by MAE, solvent to solid ratios of 10 or 20 ml/g was found to be optimum in different studies performed by MAE (Talebi et al., 2004; Pan et al., 2001; Li et al., 2004; Kwon et al., 2003; Pan et al., 2003).

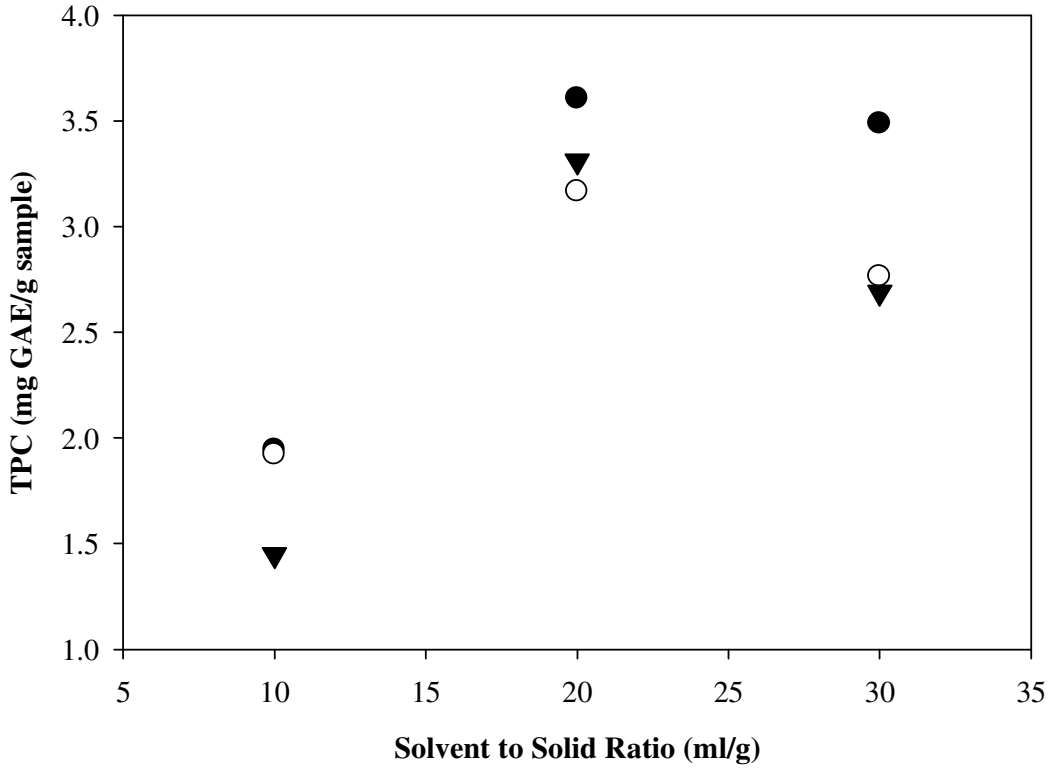


Figure 3.4. TPC of tomato pomace extracts obtained using 400 W microwave power level, different solvent to solid ratios and optimum time (●): ethanol-water for 16 min, (○): ethanol for 16 min , and (▼):water for 18 min.

Figure 3.5, 3.6 and 3.7 showed the effects of different solvent types on TPC of tomato pomace extract during MAE extraction at 700 W power. Similar to the results obtained by 400 W, ethanol-water mixture gave the highest total phenolic content followed by ethanol and then water. When ethanol-water mixture and ethanol were used, the highest total phenolic content was obtained at 14 min, whereas this time was 16 min., when water was used as a solvent.

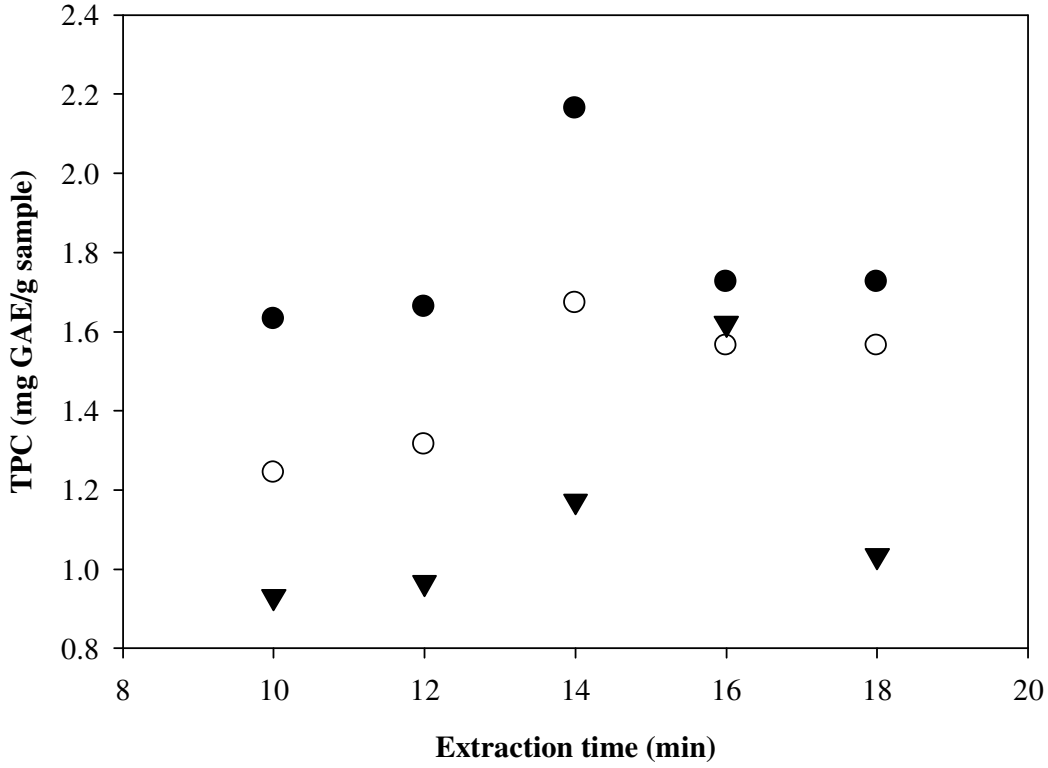


Figure 3.5. Effects of different solvent types on TPC of tomato pomace extracts obtained using solvent to solid ratio of 10 ml/g during MAE at 700 W. (●): ethanol-water, (○): ethanol, (▼): water.

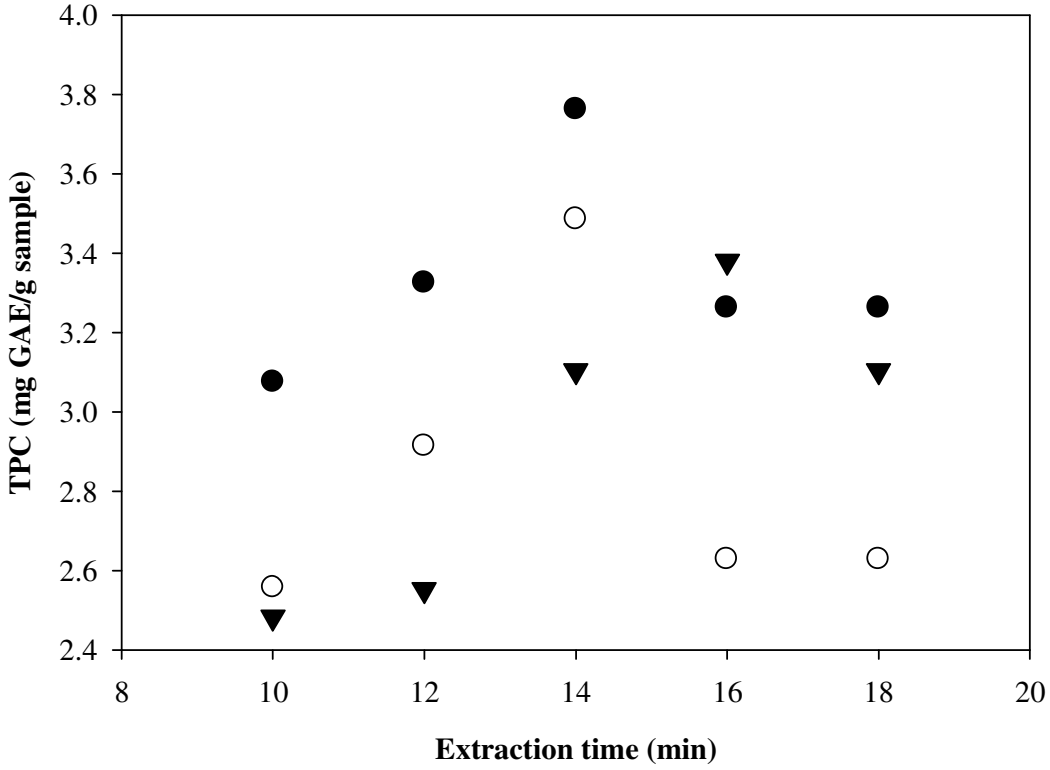


Figure 3.6. Effects of different solvent types on TPC of tomato pomace extracts obtained using solvent to solid ratio of 20 ml/g during MAE at 700 W. (●): ethanol-water, (○): ethanol, (▼): water.

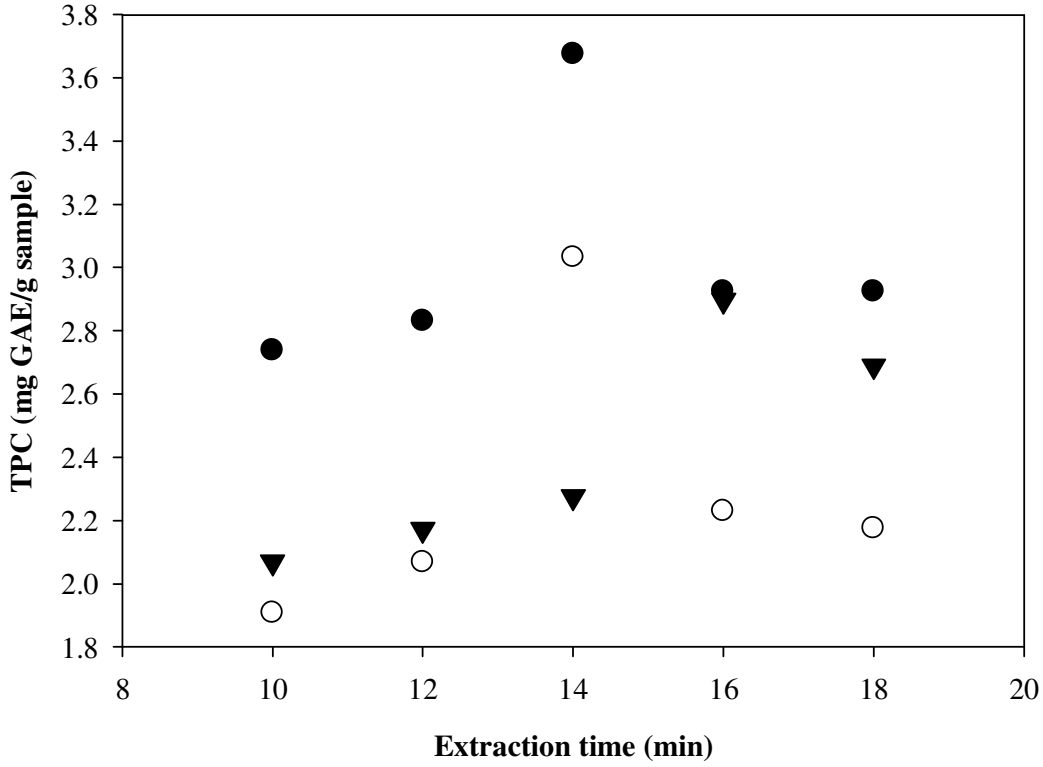


Figure 3.7. Effects of different solvent types on TPC of tomato pomace extracts obtained using solvent to solid ratio of 30 ml/g during MAE at 700 W. (●): ethanol-water, (○): ethanol, (▼): water.

TPC values obtained at 700 W were higher than the ones obtained at 400 W (Figure 3.1, 3.2, 3.3, 3.5, 3.6 and 3.7). When optimum time and the best effective extraction solvent, which was ethanol-water mixture, were considered, the amount of TPC at 400 W was 1.94, 3.61 and 3.49 mg GAE/g sample and increased to 2.16 mg GAE/g sample by 11.26%, 3.76 mg GAE/g sample by 4.35% and 3.68 mg GAE/g sample by 5.36% at 700 W with the change of solvent to solid ratio from 10 to 30 ml/g, respectively. These results are in accordance with the results of statistical analysis which shows that the power is a significant parameter ($p \leq 0.05$) (Table C2). The

increase in TPC when higher power levels were used may be due to the rapid generation of heat inside the sample with absorption of microwave energy which might lead to higher pressure gradients inside tomato pomace (Lucchesi et al., 2007). The decrease in optimum extraction time when power was increased from 400 W to 700 W (Figure 3.5-3.7) can be explained by the increase in pressure gradient in the system. This caused an increase in extraction rate. Gao et al., 2006 showed that changing power from 400 W to 1200 W decreased the extraction time of flavanoids by 45 min. They thought that cell wall rupture occurred quickly at higher power levels reaching to higher extraction temperatures in shorter time. In the study of Xiao et al. (2008), the flavonoid extraction efficiency increased with the increase in power from 200 W to 1000 W.

Similar to the results obtained at 400 W, Figure 3.8 showed that the highest TPC was determined when the solvent to solid ratio was 20 ml/g for all types of extraction solvents.

Toor and Savage (2005) studied the skin fraction of tomato cultivars for the detection of total phenolics and they stated that the hydrophenolics of those cultivars ranged from 26.9 to 30.3 mg GAE/100 g. As the tomato contains around 93% moisture, these TPC amounts could be expressed as 3.57 to 4.03 mg GAE/g dry sample. Our findings were in an agreement with their results. As expected, these results prove that some of the phenolics were lost by the removal of skins and seeds during tomato juice processing.

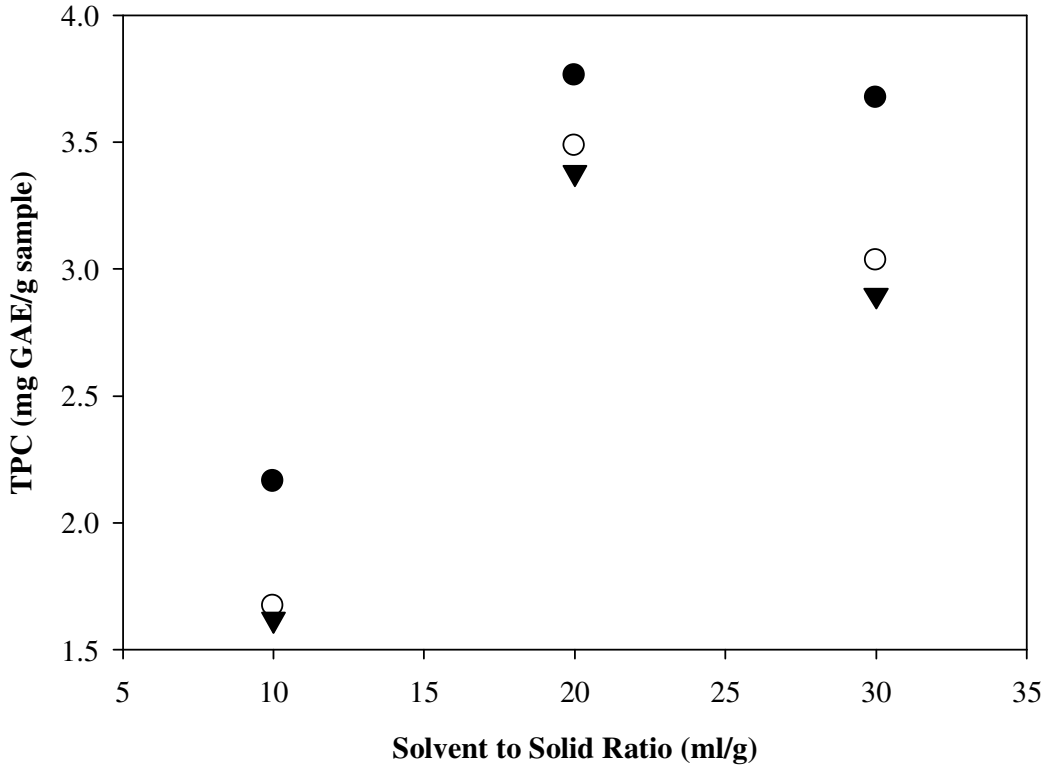


Figure 3.8. TPC of tomato pomace extracts obtained using 700 W microwave power level, different solvent to solid ratios and optimum time (●): ethanol-water for 14 min, (○): ethanol for 14 min , and (▼):water for 16 min.

As a control, conventional extraction was performed. According to preliminary experiments, the optimum time for the conventional extraction of total phenolic compounds was determined as 6 hours. Figure 3.9 shows the change of TPC of conventionally extracted tomato pomace for 6 hours when different solvent types and solvent to solid ratios were used.

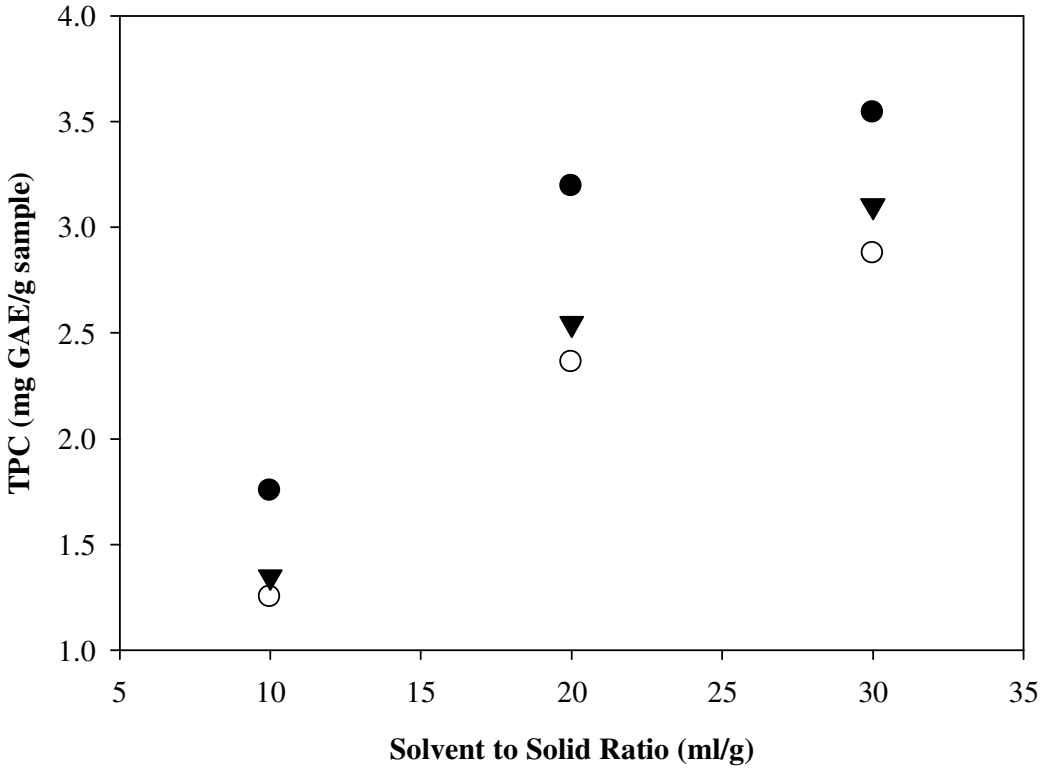


Figure 3.9. The effect of solvent to solid ratios and different solvents on TPC of tomato pomace extract obtained with conventional extraction for 6 hours. (●): ethanol-water (○): ethanol, and (▼):water.

The influence of solvent to solid ratio on TPC was found to be significant (Table C.4). As solvent to solid ratio increased, TPC increased. The highest TPC was obtained at a solvent to solid ratio of 30 ml/g. This trend was different from the one obtained in MAE. On the other hand, these findings were in agreement with several conventional extraction studies which showed that using a higher volume of solvent increased the recovery of phenolic compounds (Xiao et al., 2008; Mandal et al., 2007; Guo et al., 2001; Eskilsson and Bjorklund, 2000). Hasbay et al. (2008) reported that increasing solvent to solid ratio increased TPC for all samples due to the higher concentration gradient. Moreover, Cacace and Mazza (2003) studying mass transfer during extraction of anthocyanins from milled berries found that high solvent to solid

ratio gave higher amount of anthocyanins which could be explained by the increase in concentration gradient (the driving force during mass transfer within the solid) at a higher solvent to solid ratios.

Similar to MAE, the highest TPC was obtained with the extraction using ethanol-water mixture (Figure 3.9). The other two extraction solvent exhibited similar effect on TPC.

TPC content of the extracts obtained using MAE and conventional extraction was determined as 3.76 mg GAE/g sample at optimum solvent to solid ratio of 20 ml/g of ethanol-water mixture and at power level of 700 W. In the conventional extraction, TPC was found as 3.54 mg GAE/g sample being lower one obtained in MAE at optimum solvent to solid ratio of 30 ml/g of ethanol-water mixture. There was no significant difference between these two methods on affecting TPC content (Table C.4).

3.1.2. DPPH Radical Scavenging Activities of Tomato Pomace Extracts

Figure 3.10, 3.11 and 3.12 represent the change in antiradical efficiency of tomato pomace extracts during MAE at 400 W when different solvent types and solvent to solid ratios were used. The same extracts for the determination of TPC was used for AE analysis. Similar to TPC, AE first increased and then decreased during extraction. The reason of this decrease in AE after a certain time period could be explained by the decomposition of some antioxidant compounds showing low heat tolerance due to the overexposure of samples to microwaves. While the extracts of ethanol-water and ethanol gave the highest AE at 16 min, the highest AE value was obtained at 18 min when water was used as a solvent. The highest values of AE was determined as 4.17, 4.52 and 4.18 mg DPPH/ g sample for ethanol-water mixture for solvent to solid ratio of 10, 20 and 30 ml/g, respectively (Figure 3.10, 3.11 and 3.12). The effect of extraction time was found to be statistically significant (Table C.5). The solvent types were also found to be effective on AE of tomato pomace extract (Table C.5).

According to Tukey test, solvents differed from each other in affecting AE. These findings were similar to TPC results. The extracts containing the highest TPC had the highest AE too.

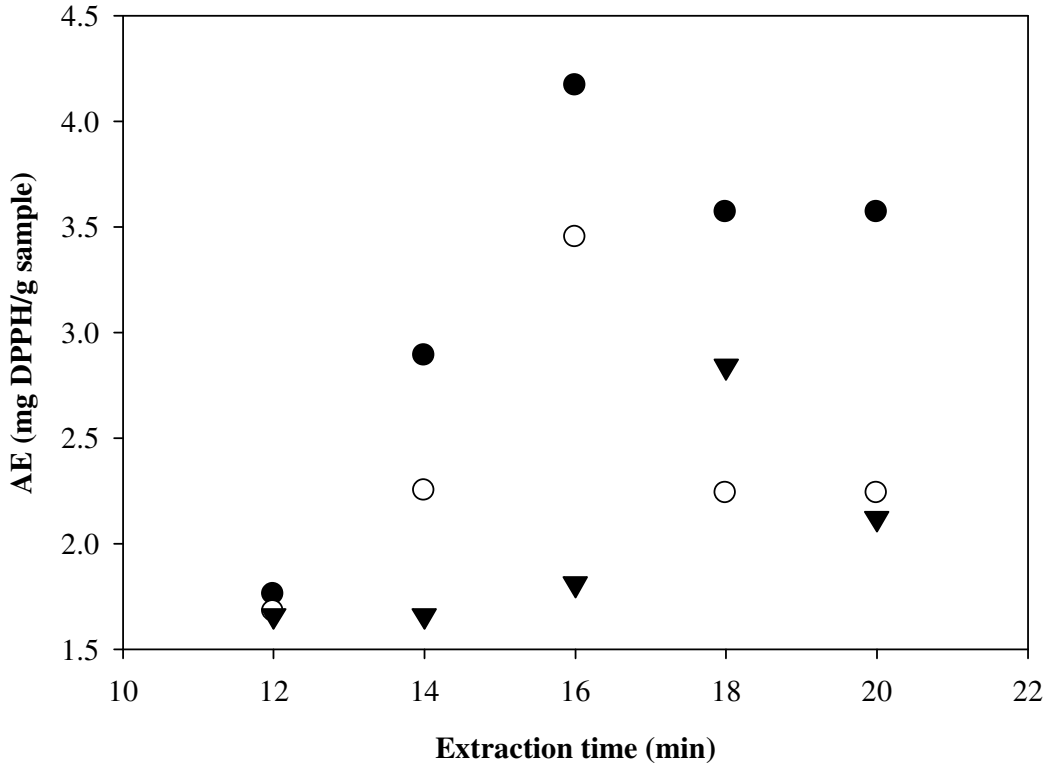


Figure 3.10. Effects of different solvent types on AE of tomato pomace extracts obtained using solvent to solid ratio of 10 ml/g during MAE at 400 W. (●): ethanol-water, (○): ethanol, (▼): water.

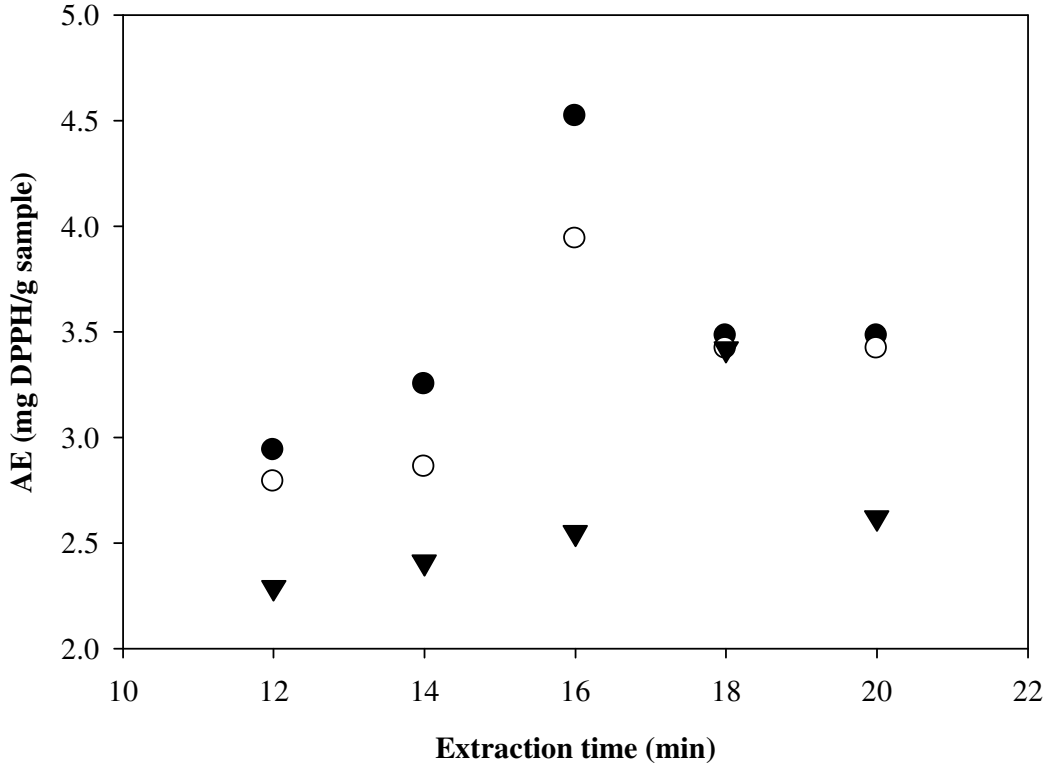


Figure 3.11. Effects of different solvent types on AE of tomato pomace extracts obtained using solvent to solid ratio of 20 ml/g during MAE at 400 W. (●): ethanol-water, (○): ethanol, (▼): water.

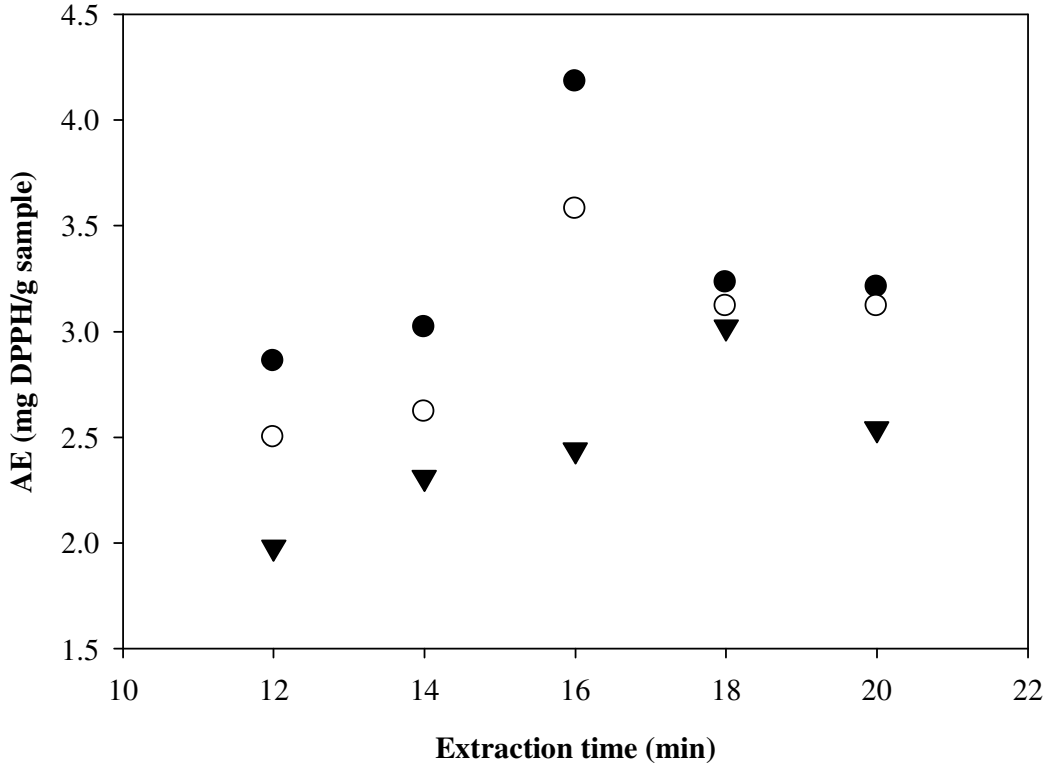


Figure 3.12. Effects of different solvent types on AE of tomato pomace extracts obtained using solvent to solid ratio of 30 ml/g during MAE at 400 W. (●): ethanol-water, (○): ethanol, (▼): water.

Figure 3.13 showed that AE values were affected by the solvent to solid ratio and the highest AE was observed at the ratio of 20 ml/g. AE value decreased when a higher solvent to solid ratio (30 ml/g) was used for all solvents. According to ANOVA analysis, solvent to solid ratio was found to be a significant parameter in affecting AE (Table C5). Solvent to solid ratio of 10 ml/g was found to be significantly different than the others. The findings were in accordance with the optimum solvent to solid ratio determined in TPC.

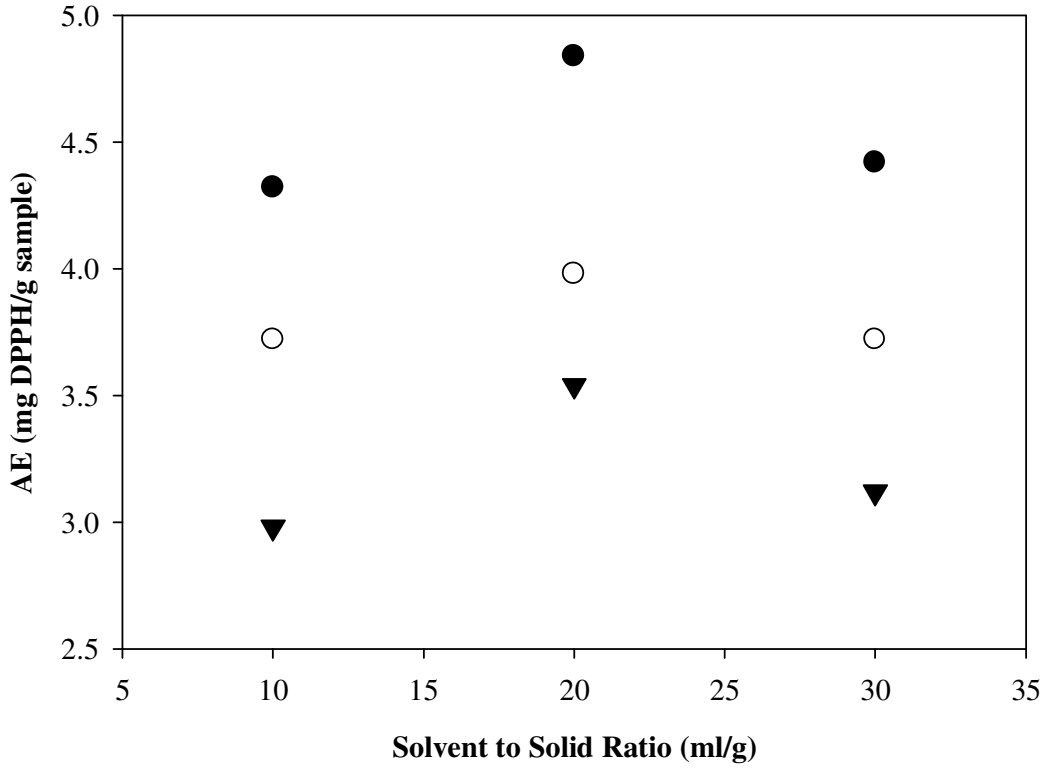


Figure 3.13. AE of tomato pomace extracts obtained using 400 W microwave power level, different solvent to solid ratios and optimum time (●): ethanol-water for 16 min, (○): ethanol for 16 min , and (▼): water for 16 min.

As can be seen in Figure 3.14, 3.15 and 3.16, the highest AE was obtained by ethanol-water mixture when the power increased to 700 W. The increase in power from 400 W to 700 W shortened the extraction time by 2 min and increased AE by 7.07%. Power was found to be statistically important as shown Table C.5. Figure 3.17 shows that the effect of solvent to solid ratio on AE of tomato pomace extracts at 700 W. The highest AE was determined as 4.84 mg DPPH/g sample at the optimum solvent to solid ratio of 20 ml/g of ethanol-water mixture.

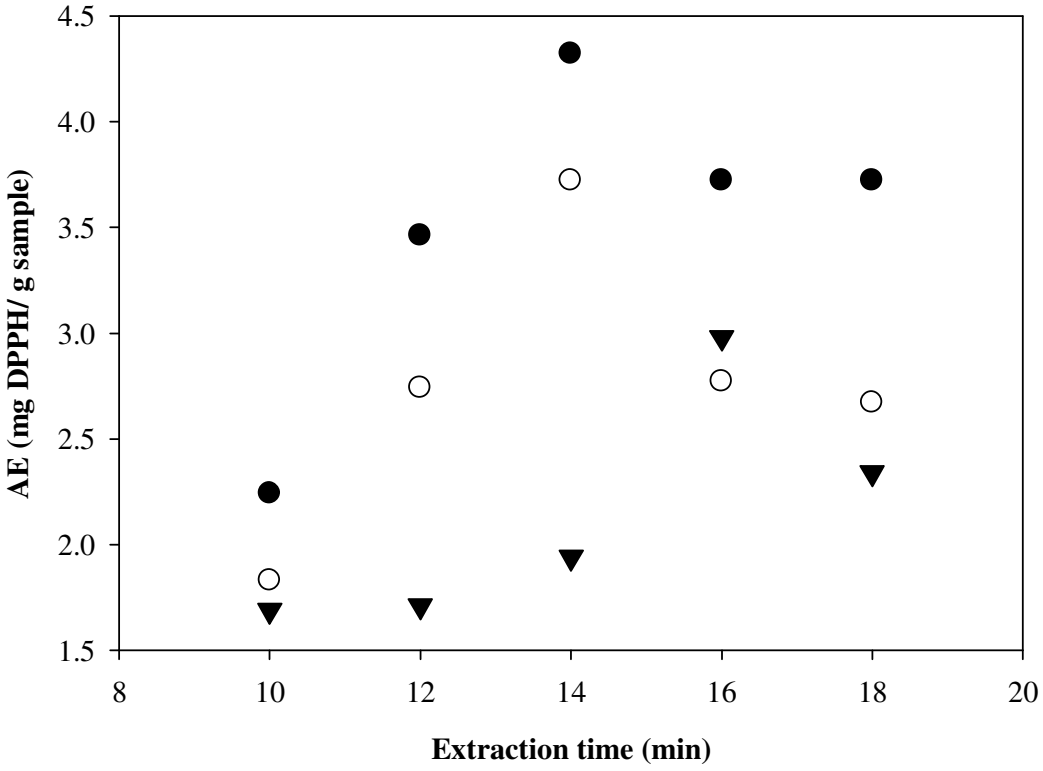


Figure 3.14. Effects of different solvent types on AE of tomato pomace extracts obtained using solvent to solid ratio of 10 ml/g during MAE at 700 W. (●): ethanol-water, (○): ethanol, (▼): water.

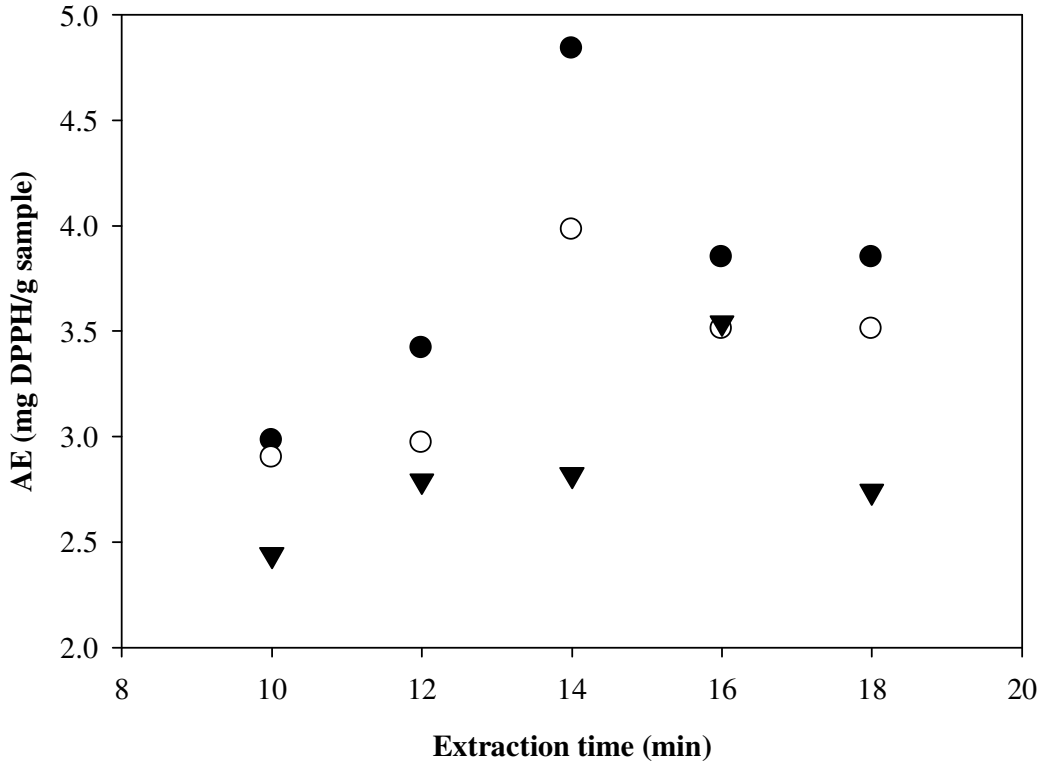


Figure 3.15. Effects of different solvent types on AE of tomato pomace extracts obtained using solvent to solid ratio of 20 ml/g during MAE at 700 W. (●): ethanol-water, (○): ethanol, (▼): water.

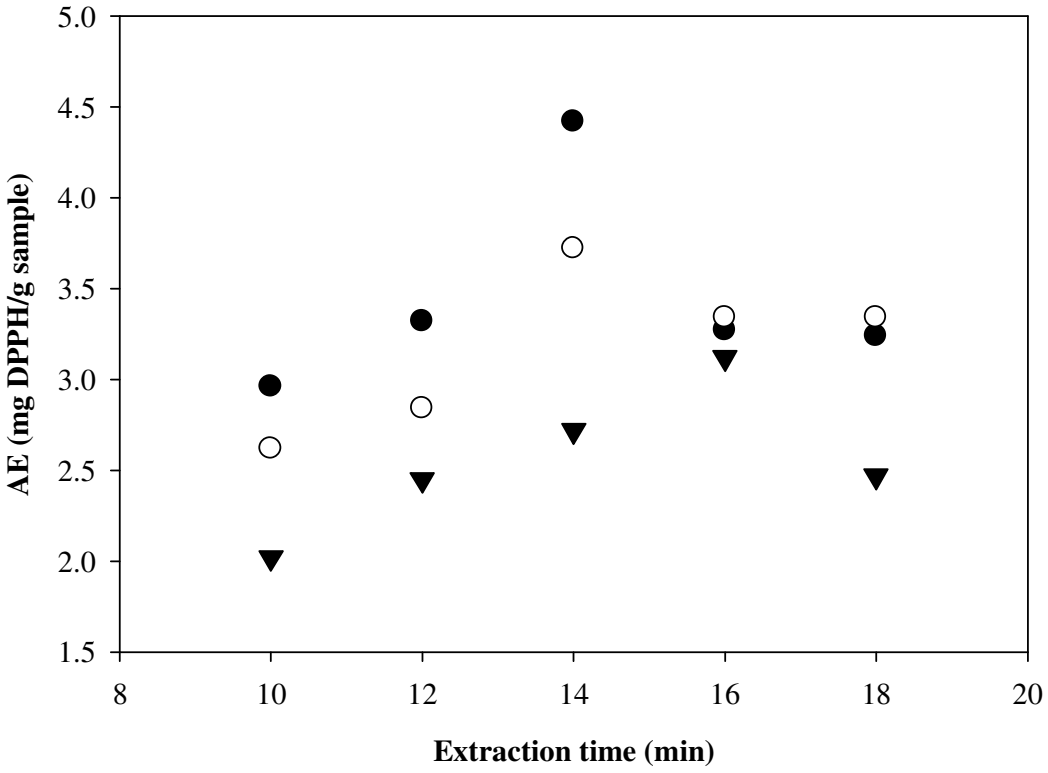


Figure 3.16. Effects of different solvent types on AE of tomato pomace extracts obtained using solvent to solid ratio of 30 ml/g during MAE at 700 W. (●): ethanol-water, (○): ethanol, (▼): water.

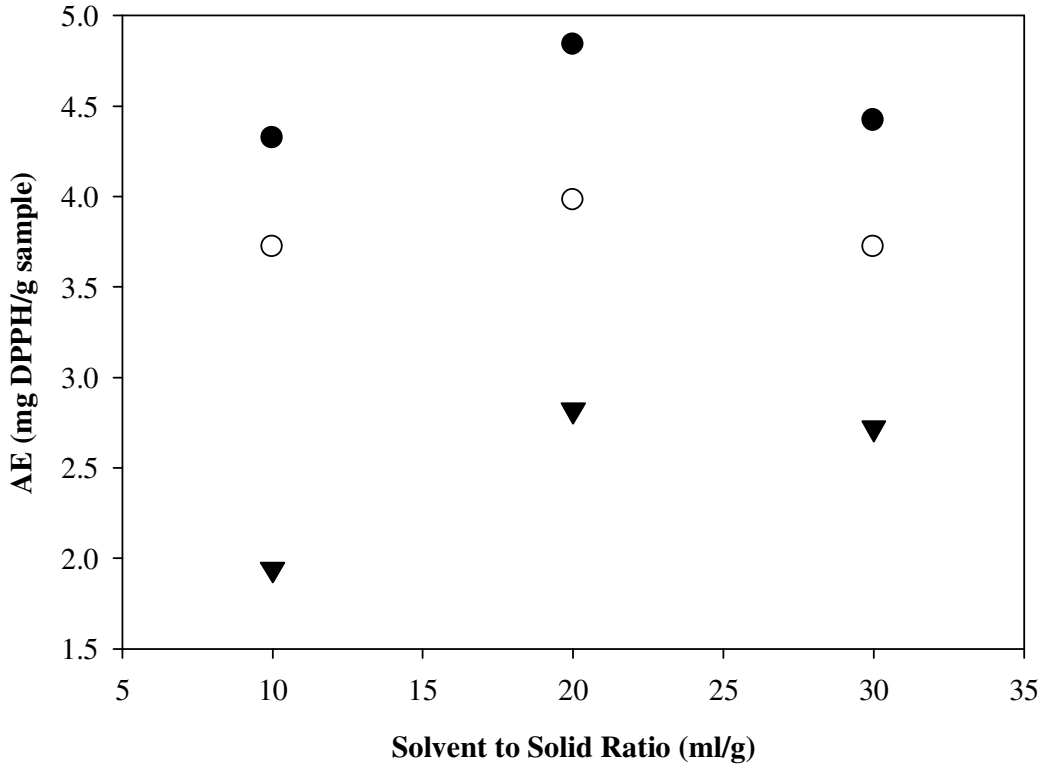


Figure 3.17. AE of tomato pomace extracts obtained using 700 W microwave power level, different solvent to solid ratios and optimum time (●): ethanol-water for 14 min, (○): ethanol for 14 min , and (▼): water for 14 min.

As can be seen in Figure 3.18, the increase in solvent to solid ratio in conventional extraction increased AE. When solvent to solid ratio was 30 mg/l, AE was determined as 4.25, 3.68 and 3.25 for ethanol-water, ethanol and water, respectively. According to statistical analysis all solvent types were different from each other, and the solvent used to obtain extract with significantly higher AE was ethanol-water mixture. Similar to TPC, the lowest solvent to solid ratio was found to provide an extract with significantly lower AE. Extraction method was found to affect AE of extracts significantly. AE of tomato extracts obtained by MAE were found to be higher than the ones obtained by conventional extraction. This can be due to longer

extraction time of conventional extraction as compared to MAE. Exposing heat during longer time may cause some losses of antioxidant compounds. Therefore, decreasing extraction time could provide better extraction of antioxidant compounds which are liable to decomposition with heat and oxygen.

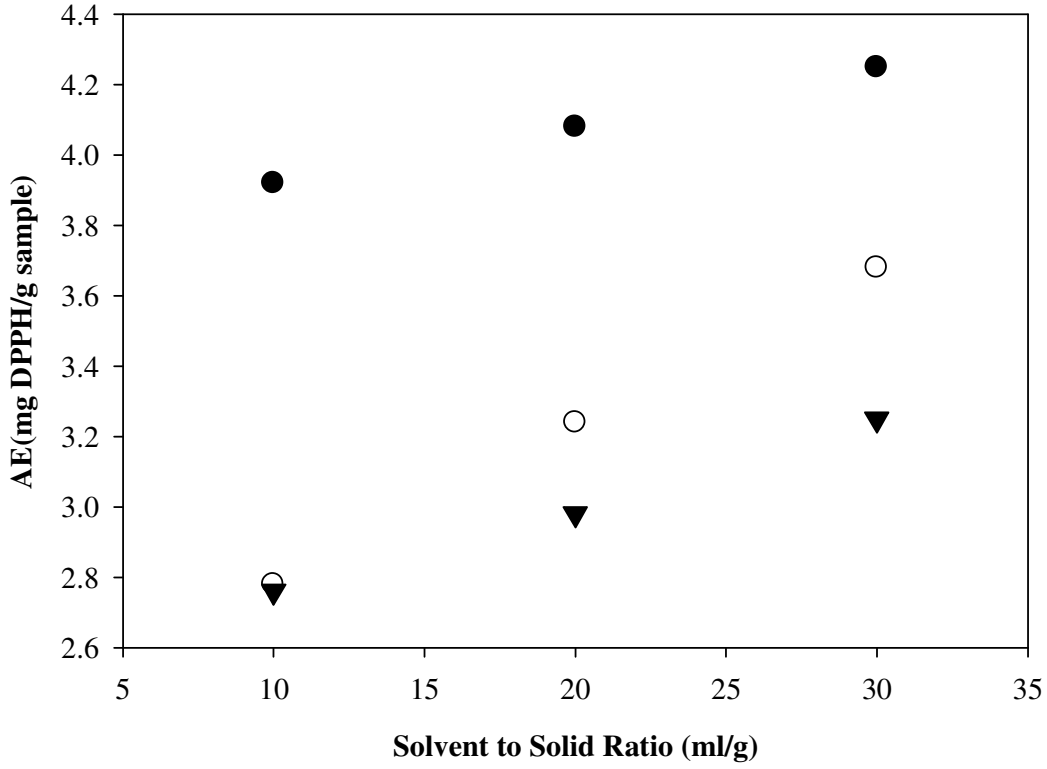


Figure 3.18. The effect of solvent to solid ratios and different solvents on AE of tomato pomace extract obtained with conventional extraction for 6 hours. (●): ethanol-water (○): ethanol, and (▼):water.

3.1.3. Concentration of Phenolic Acids of Tomato Pomace Extracts Using HPLC

For the identification of phenolic compounds, the extracts obtained at 20 ml/g solvent to solid ratio for both extraction methods were analyzed by HPLC. For MAE, the extracts obtained at 700 W and 14 min of extraction time using ethanol-water and

ethanol and at 700 W and 16 min water were analyzed by HPLC. According to Table 3.1., it can be seen that the main compounds in tomato extract were vannilic and gentisic acid. They were followed by epicatechin, quercitrin and syringic acid. No gallic acid was determined in the extractas. MAE resulted in higher concentrations of these compounds. This could be due to comparatively shorter operation time used in MAE than in conventional extraction. The highest concentration of main phenolics was obtained when ethanol-water mixture was used as a solvent.

Table 3.1 Concentration of main phenolic acids in tomato pomace extracts (mg/g sample).

Extraction Method	Solvent Type	Vanillic	Epicatechin	Syringic	Gentisic	Quercitin
MAE	Ethanol-Water	0.0052	0.0023	0.0004	0.0077	0.0006
MAE	Ethanol	0.0049	0.0007	0.0022	0.0050	0.0003
MAE	Water	0.0019	-	0.0018	0.0013	-
Conventional	Ethanol-Water	0.0012	0.0011	0.0037	0.0064	0.0001
Conventional	Ethanol	0.0023	0.0011	0.0023	-	0.0002
Conventional	Water	0.0012	0.0005	0.0009	-	0.0001

3.2. Extraction of Phenolic Compounds from Sour Cherry Pomace

In this study, the effects of different microwave power levels (400 W and 700 W), solvent types (water, ethanol and ethanol-water mixture), extraction times (10, 12, 14 and 16 min) and solvent to solid ratios (10, 20 and 30 ml/g) on TPC, AE and concentration of major phenolic compounds obtained by MAE were studied. In addition, MAE was compared with conventional extraction. Experimental data are shown in Appendix B (Table B.3 and B.4). Moreover, moisture content of tomato pomace was found as 13.75%.

3.2.1. Total Phenolic Content of Sour Cherry Pomace Extracts

Figure 3.19, 3.20 and 3.21 indicate the change of TPC of sour cherry pomace extracts obtained with different solvent types and different solvent to solid ratios during MAE at 400 W. Time was found to be a significant factor in affecting TPC (Table C.8). During extraction, TPC values increased with the increase in extraction time, reached to maximum at 14 min, and then decreased. As explained in section 3.1.1., longer extraction time might cause the destruction of phenolic compounds.

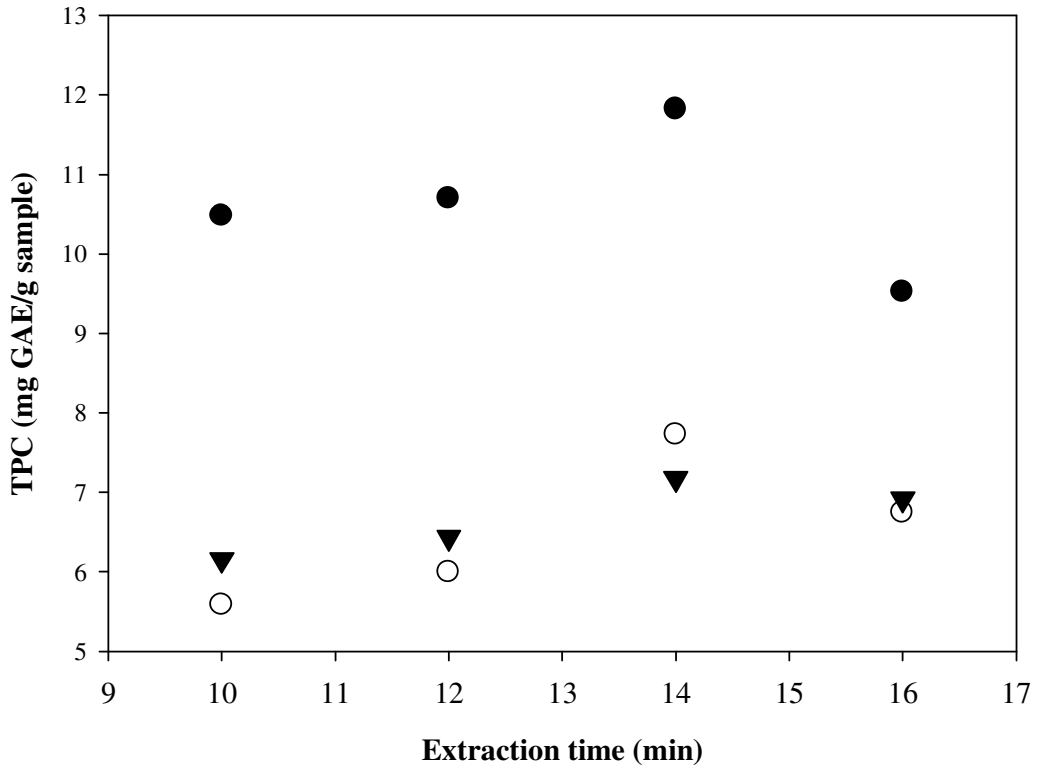


Figure 3.19. Effects of different solvent types on TPC of sour cherry pomace extracts obtained using solvent to solid ratio of 10 ml/g during MAE at 400 W. (●): ethanol-water, (○): ethanol, (▼): water.

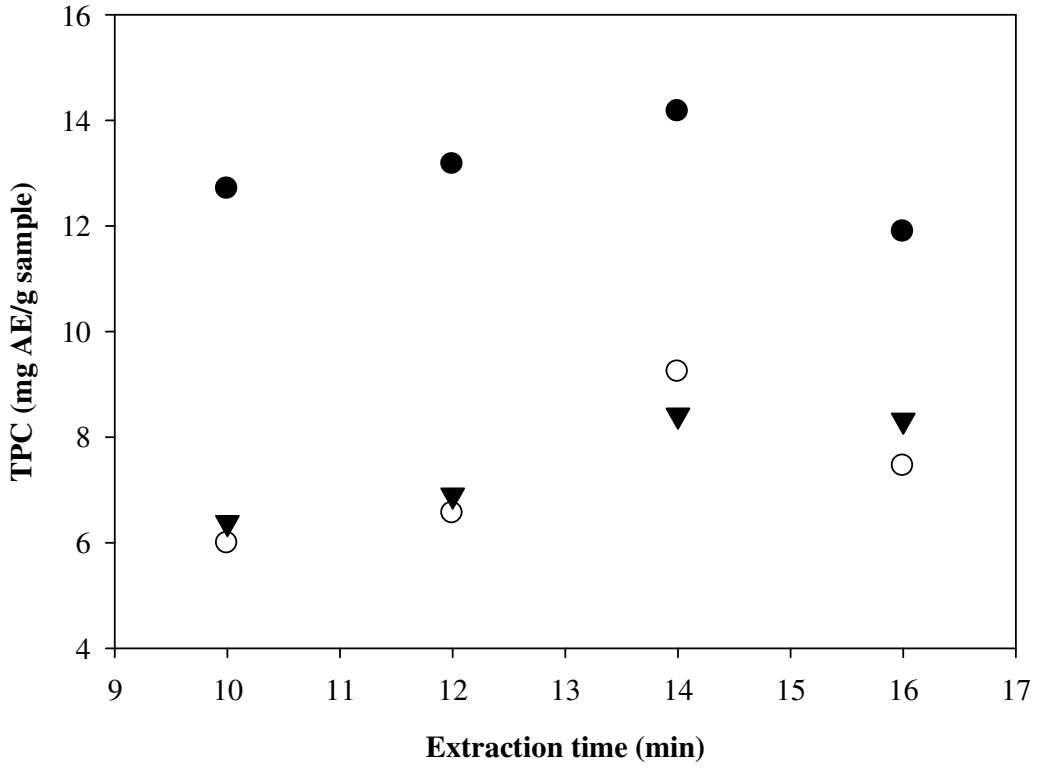


Figure 3.20. Effects of different solvent types on TPC of sour cherry pomace extracts obtained using solvent to solid ratio of 20 ml/g during MAE at 400 W. (●): ethanol-water, (○): ethanol, (▼): water.

The effects of solvent type on TPC of sour cherry pomace are different from the results found for tomato pomace. In sour cherry pomace extracts, the optimum time to reach the highest TPC was the same for all solvent types. However, in tomato pomace extracts, water was found to extend the extraction time for 2 min as compared to other solvents. Ethanol-water mixture was found to be the most effective solvent type as in the extraction of phenolic compounds from tomato pomace due to its higher dissipation factor. There were significant differences between solvent types (Table C.8).

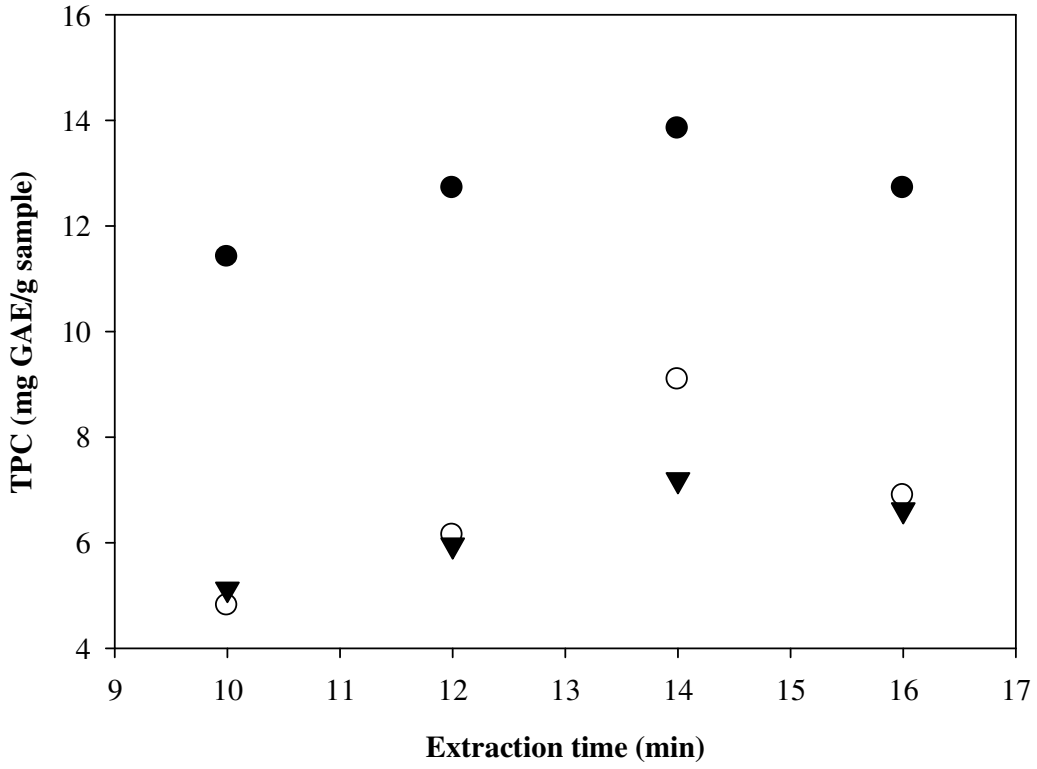


Figure 3.21. Effects of different solvent types on TPC of sour cherry pomace extracts obtained using solvent to solid ratio of 30 ml/g during MAE at 400 W. (●): ethanol-water, (○): ethanol, (▼): water.

The effect of solvent to solid ratio on TPC of sour cherry pomace extracts was demostrated in Figure 3.22. It was found that the highest TPC values were obtained at a ratio of 20 ml/g and then decreased slightly similar to the trend observed for tomato pomace extracts. When the data were evaluated statistically, there was no significant difference between 20 and 30 ml/g of solvent to solid ratios which were higher than 10 ml/g.

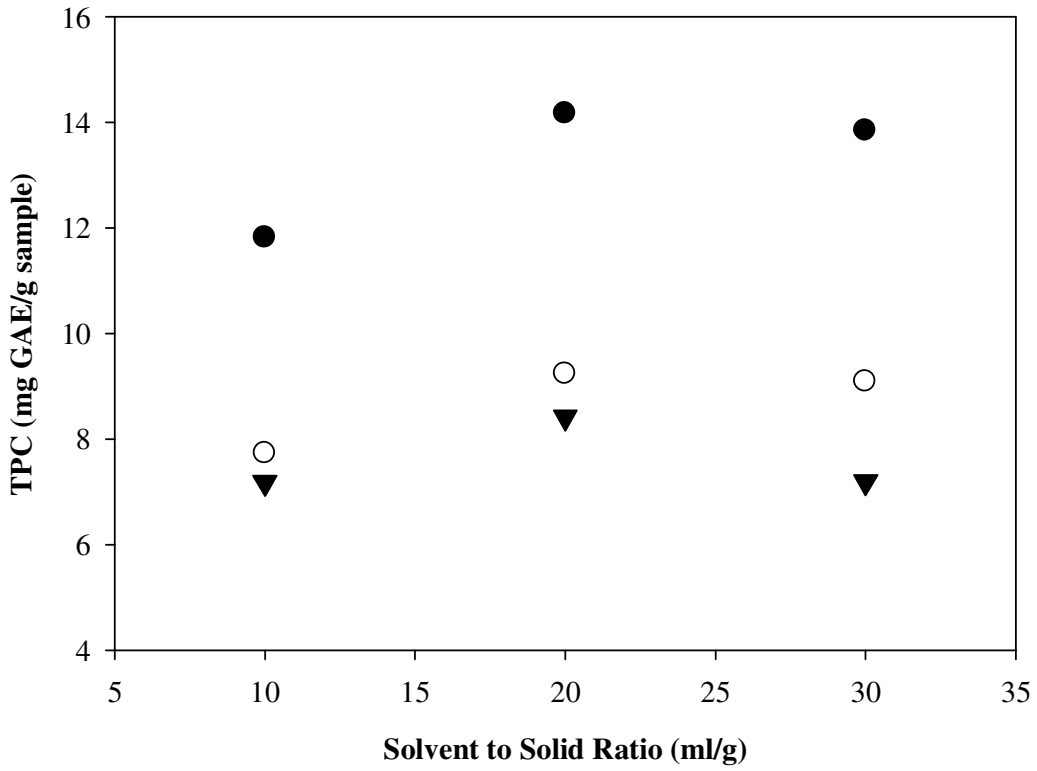


Figure 3.22. TPC of sour cherry pomace extracts obtained using 400 W microwave power level, different solvent to solid ratios and optimum time (●): ethanol-water for 14 min, (○): ethanol for 14 min , and (▼): water for 14 min.

Figure 3.23, 3.24 and 3.25, showed that the highest TPC in sour cherry phenolic content was obtained when ethanol-water was used as a solvent. The increase in power from 400 W to 700 W enhanced TPC (Figure 3.23-3.25). Power increase also shortened the extraction time for 2 minutes.

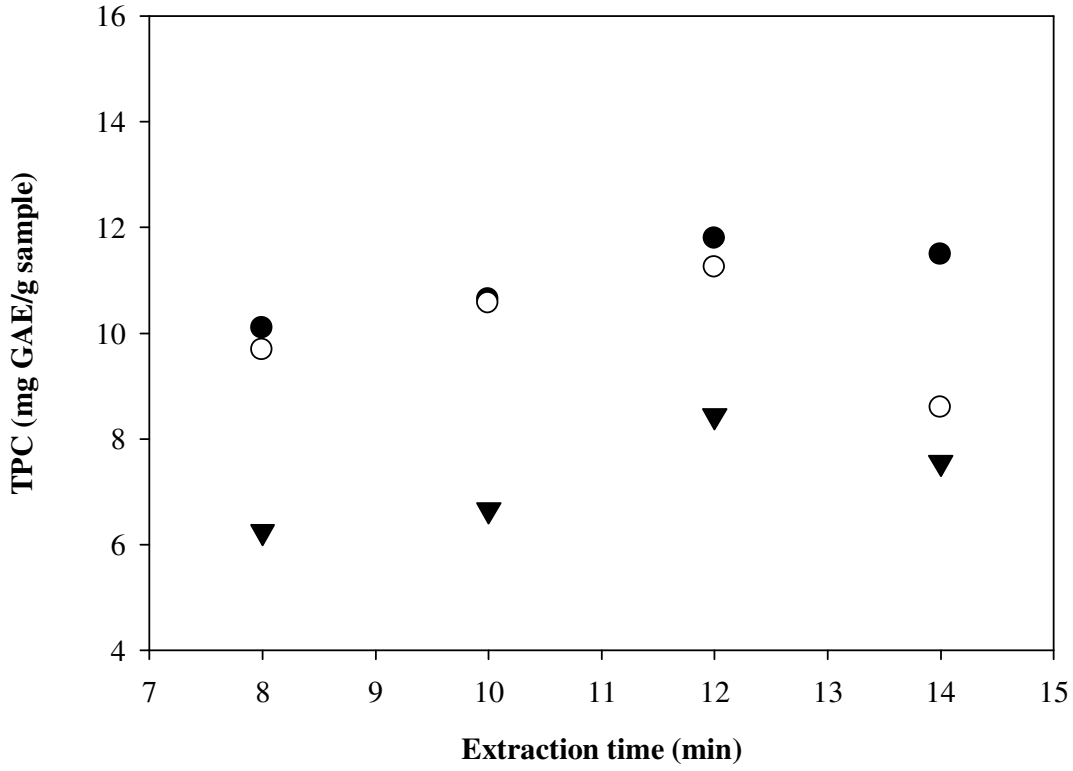


Figure 3.23. Effects of different solvent types on TPC of sour cherry pomace extracts obtained using solvent to solid ratio of 10 ml/g during MAE at 700 W. (●): ethanol-water, (○): ethanol, (▼): water.

Kim et al. (2005) reported that TPC for sour cherries changed between 8.51 and 16.44 mg GAE/g sample. In addition, Chaovanalikit and Wrolstad (2004) examined total phenolic content of different parts (skins, flesh and pits) of a sour cherry. TPC in skin part was 3.33 ± 0.41 mg GAE/fresh weight. Converting this result to dry basis using the moisture content of sour cherry 81%, TPC would be 14.48 mg GAE/g dry sample. Similary, our findings which are ranged between 5.12 and 14.17 mg GAE/g sample was found to be consistent with this study. However, the results of Hasbay et al. (2008) were much lower than our findings. This difference may be due to the type of fresh sour cherry pomace, extraction method and storage conditions

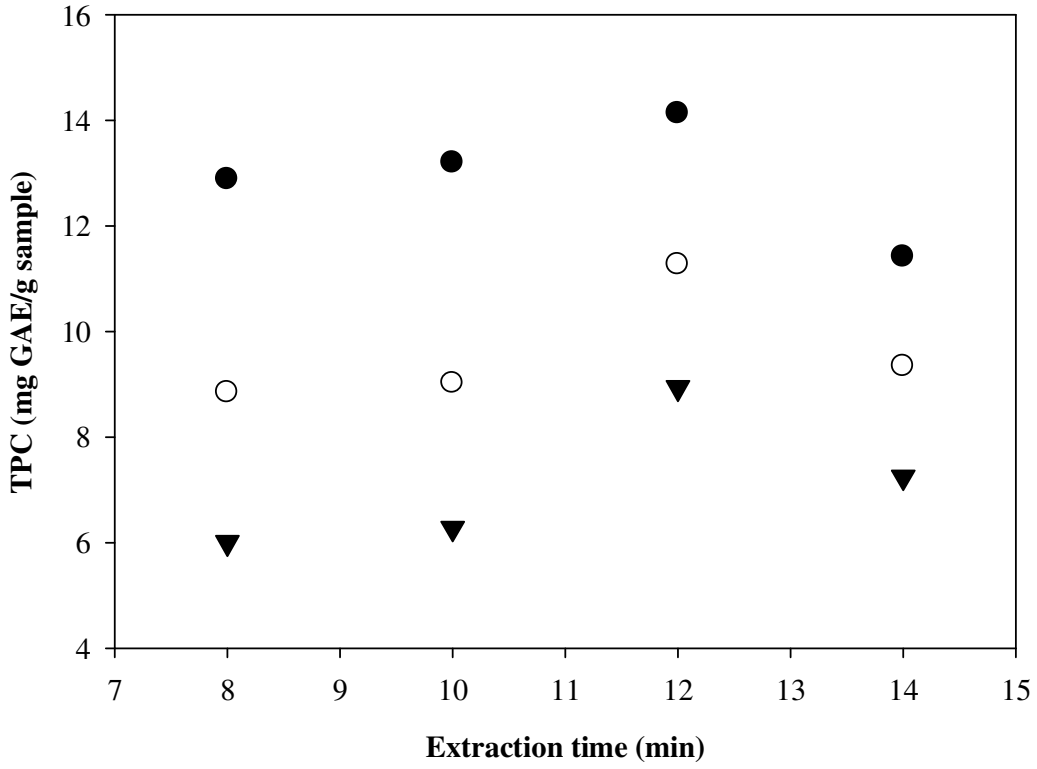


Figure 3.24. Effects of different solvent types on TPC of sour cherry pomace extracts obtained using solvent to solid ratio of 20 ml/g during MAE at 700 W. (●): ethanol-water, (○): ethanol, (▼): water.

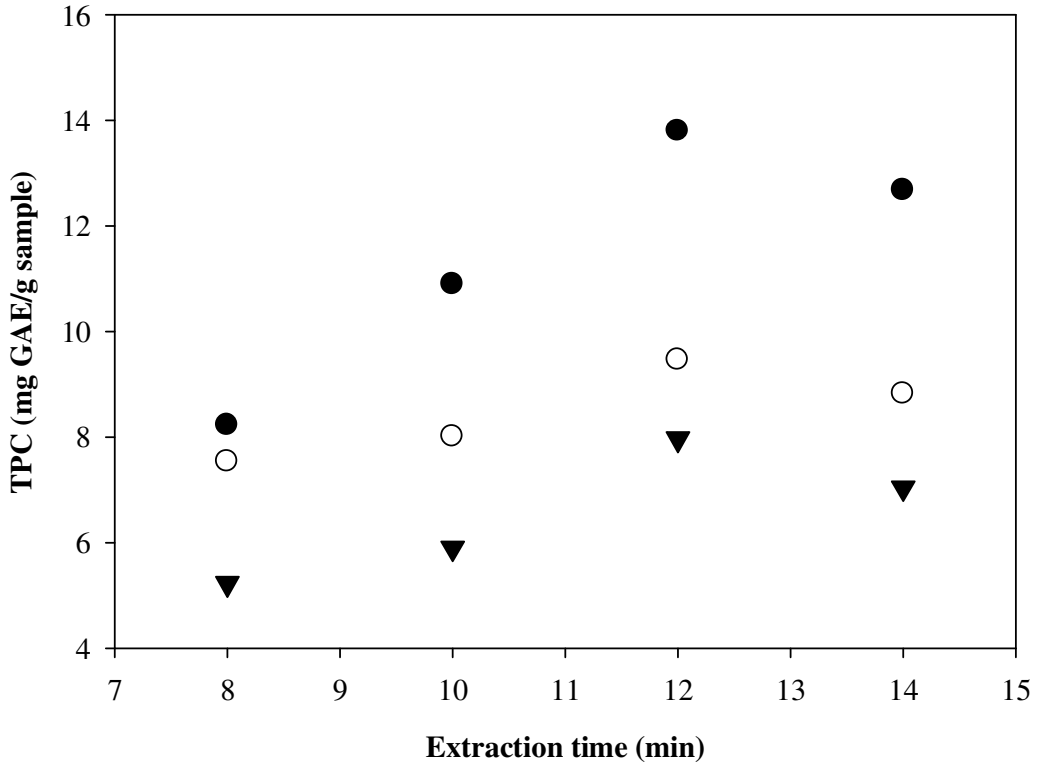


Figure 3.25. Effects of different solvent types on TPC of sour cherry pomace extracts obtained using solvent to solid ratio of 30 ml/g during MAE at 700 W. (●): ethanol-water, (○): ethanol, (▼): water.

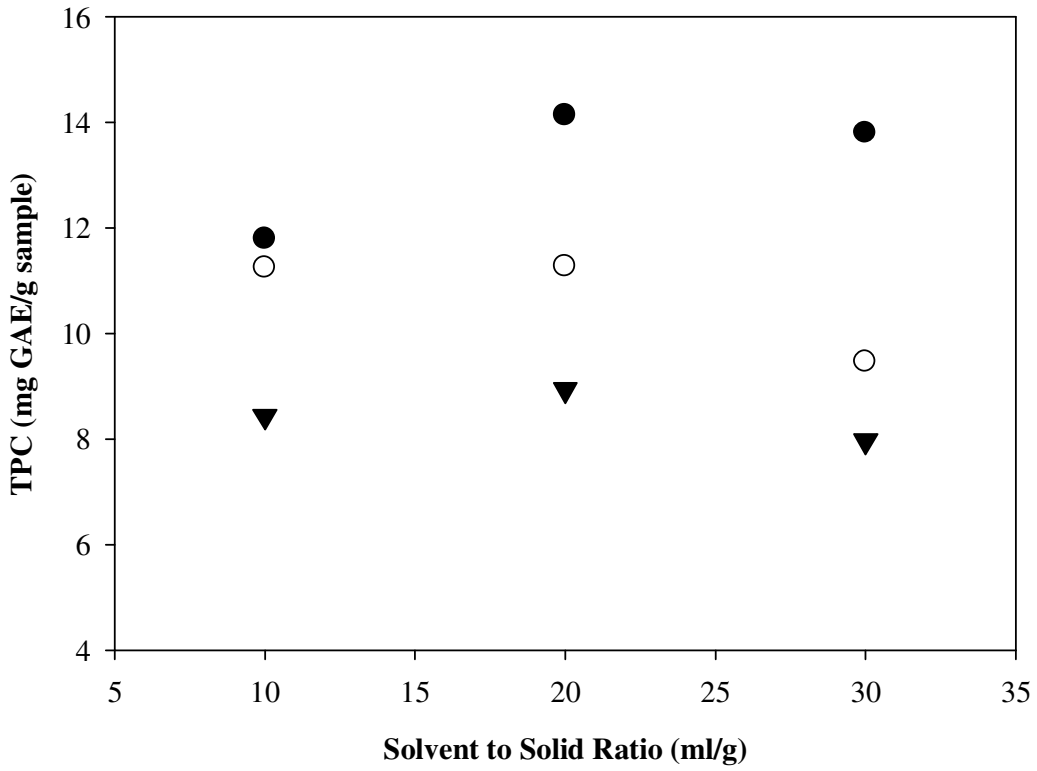


Figure 3.26. TPC of sour cherry pomace extracts obtained using 700 W microwave power level, different solvent to solid ratios and optimum time (●): ethanol-water for 12 min, (○): ethanol for 12 min , and (▼): water for 12 min.

Figure 3.26 shows the effect of solvent to solid ratio on TPC when the extractions were performed at 700 W power level. The highest TPC was determined as 14.14 mg GAE/g sample at the solvent to solid ratio of 20 ml/g.

Figure 3.27 shows the change of TPC with different solvent to solid ratios in the sour cherry pomace extracts obtained by conventional extraction. The preliminary experiments indicated that the best time for extraction of total phenolic compounds was determined as 6 hours. As can be seen in Figure 3.27, 13.78, 8.09 and 10.85 mg GAE/g sample were obtained at 30 ml/g solvent to solid ratio with ethanol-water mixture, ethanol and water, respectively. Ethanol-water mixture and 30 ml/g of

solvent to solid ratio were found to be the most effective combination on extraction of TPC. Similar to tomato pomace results, the extraction method was not significant on TPC of sour cherry pomace extracts as can be in Appendix C (Table C.9).

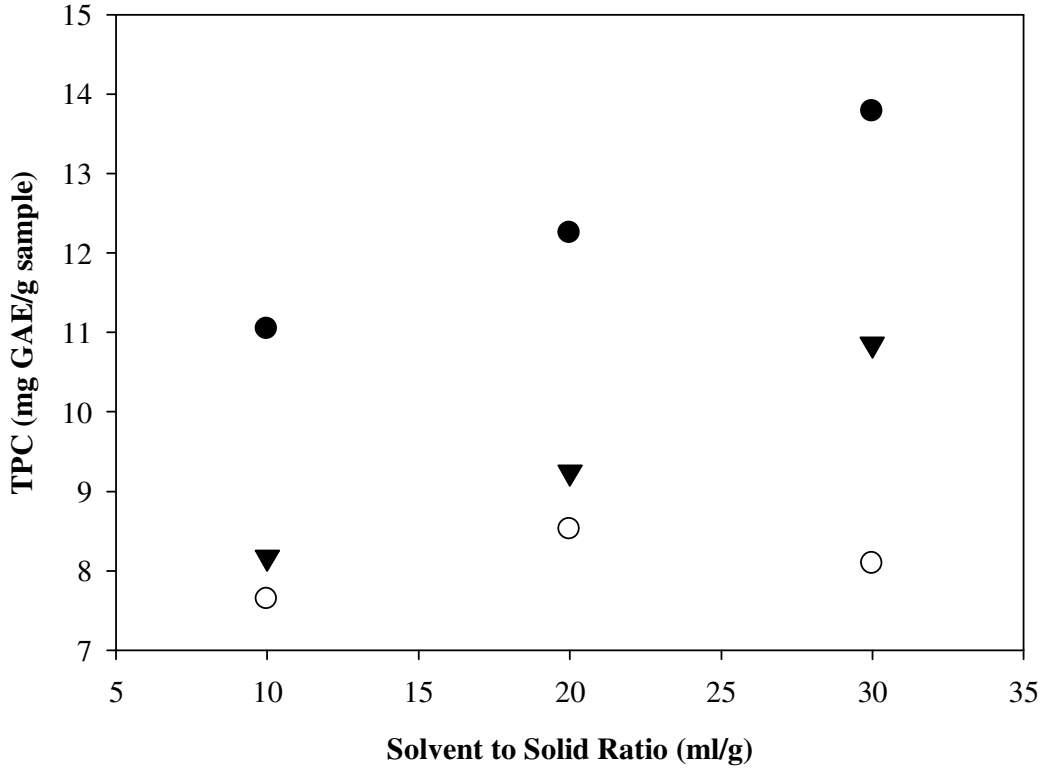


Figure 3.27. The effect of solvent to solid ratios and different solvents on TPC of sour cherry pomace extract obtained with conventional extraction for 6 hours. (●): ethanol-water (○): ethanol, and (▼): water.

3.2.2. DPPH Radical Scavenging Activities of Sour Cherry Pomace Extracts

According to ANOVA results, the effects of extraction time, solvent type, solvent to solid ratio and microwave power on AE were found to be significant as can be seen in Appendix C (Table C.10). Figure 3.28, 3.29 and 3.30 represent the change in antiradical efficiency of sour cherry pomace extracts during MAE at 400 W. AE values were evaluated for the extracts obtained at different extraction times (10, 12,

14 and 16) under 400 W. The highest values of AE was determined as 24.76, 28.32 and 27.98 mg DPPH/ g sample for ethanol-water mixture as can be seen in Figure 3.28, 3.29 and 3.30, respectively.

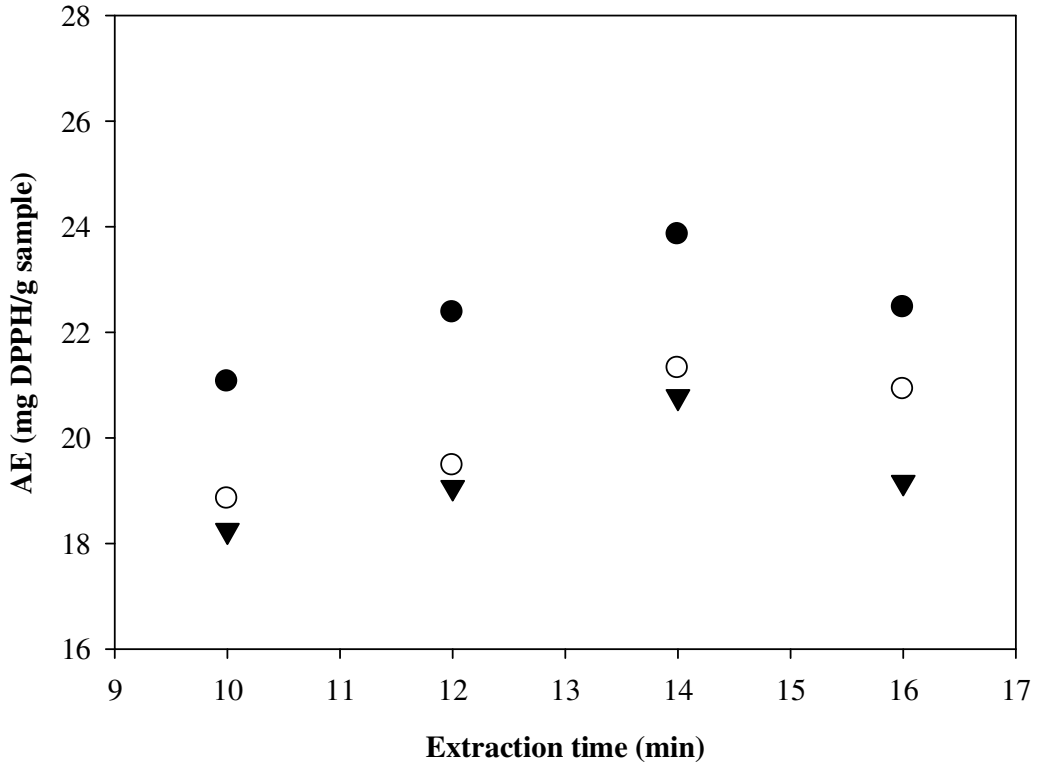


Figure 3.28. Effects of different solvent types on AE of sour cherry pomace extracts obtained using solvent to solid ratio of 10 ml/g during MAE at 400 W. (●): ethanol-water, (○): ethanol, (▼): water.

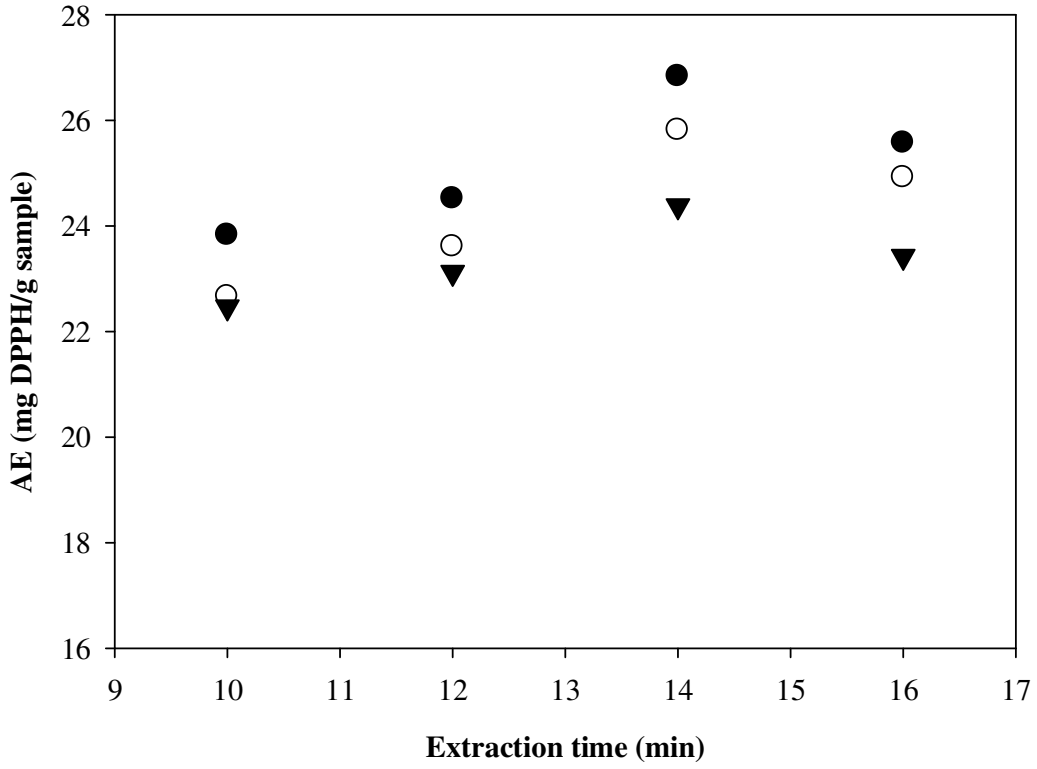


Figure 3.29. Effects of different solvent types on AE of sour cherry pomace extracts obtained using solvent to solid ratio of 20 ml/g during MAE at 400 W. (●): ethanol-water, (○): ethanol, (▼): water.

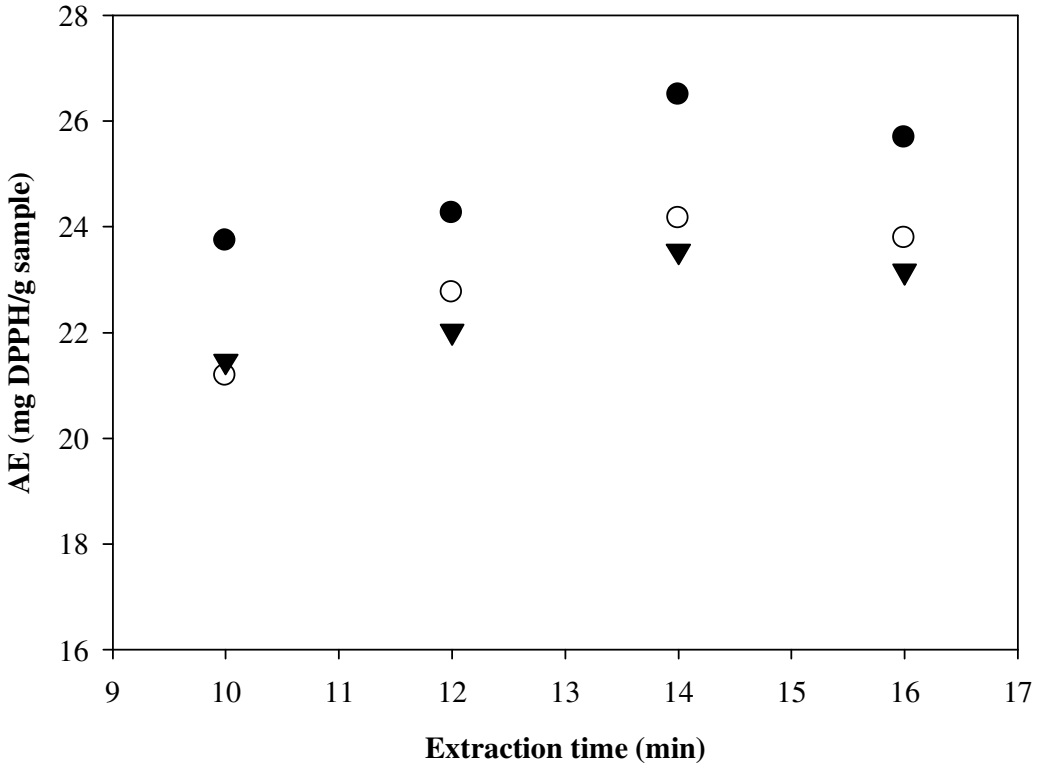


Figure 3.30. Effects of different solvent types on AE of sour cherry pomace extracts obtained using solvent to solid ratio of 30 ml/g during MAE at 400 W. (●): ethanol-water, (○): ethanol, (▼): water.

The solvent types were determined to be significantly effective on AE of sour cherry pomace extracts ($p \leq 0.05$). All types of extraction solvents differed from each other according to Tukey test, and ethanol-water mixture had the highest significance among them. As can be seen from Figure 3.31, solvent to solid ratio of 20 ml/ gave the best antiradical efficieny and increasing solvent to solid ratio further decreased AE slightly. These findings were supported by statistical analysis, in that, there was significant difference between different ratios.

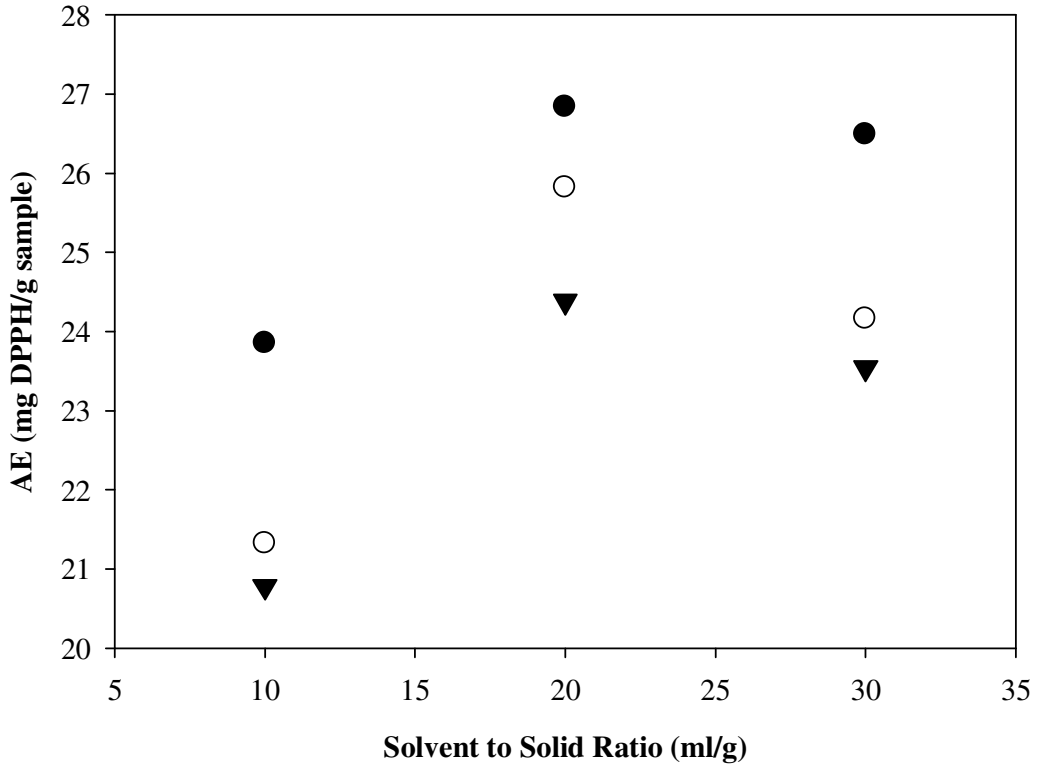


Figure 3.31. AE of sour cherry pomace extracts obtained using 400 W microwave power level, different solvent to solid ratios and optimum time (●): ethanol-water for 14 min, (○): ethanol for 14 min , and (▼):water for 14 min.

Figure 3.32, 3.33 and 3.34, showed that the highest AE of sour cherry pomace was obtained when ethanol-water mixture was used. The optimum extraction time decreased with the increase in power. At the optimum conditions of 20 ml/g solvent to solid ratio and ethanol-water mixture, the increase in power from 400 W to 700 W increased AE by 5.15%.

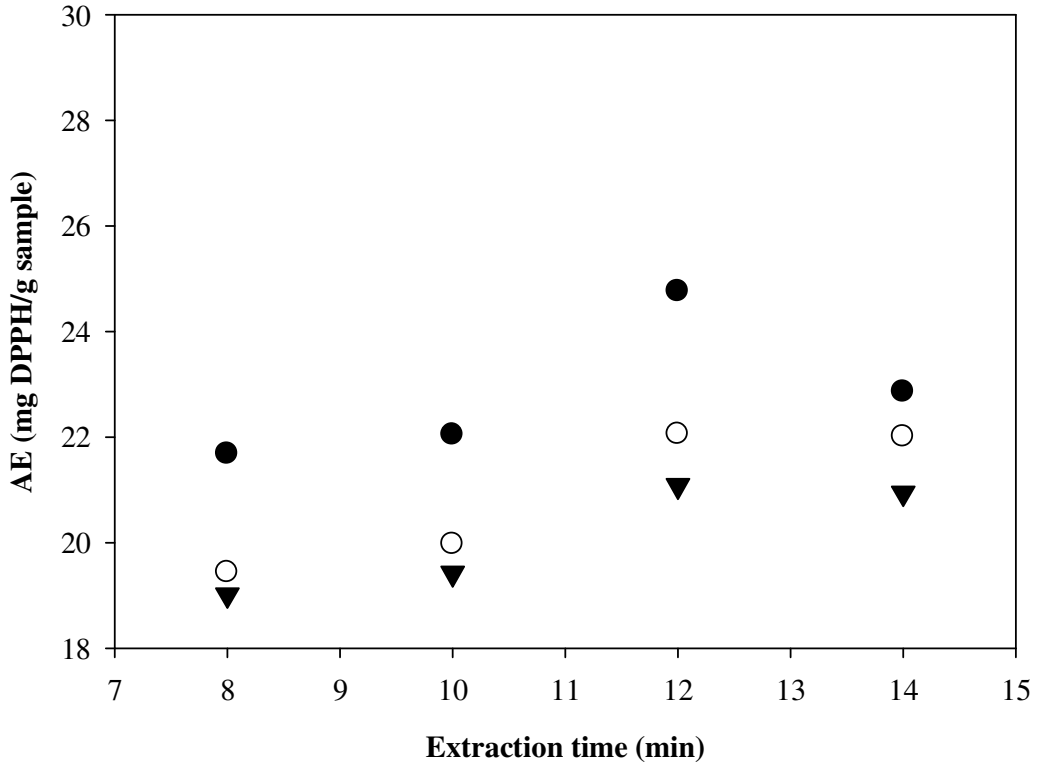


Figure 3.32. Effects of different solvent types on AE of sour cherry pomace extracts obtained using solvent to solid ratio of 10 ml/g during MAE at 700 W. (●): ethanol-water, (○): ethanol, (▼): water.

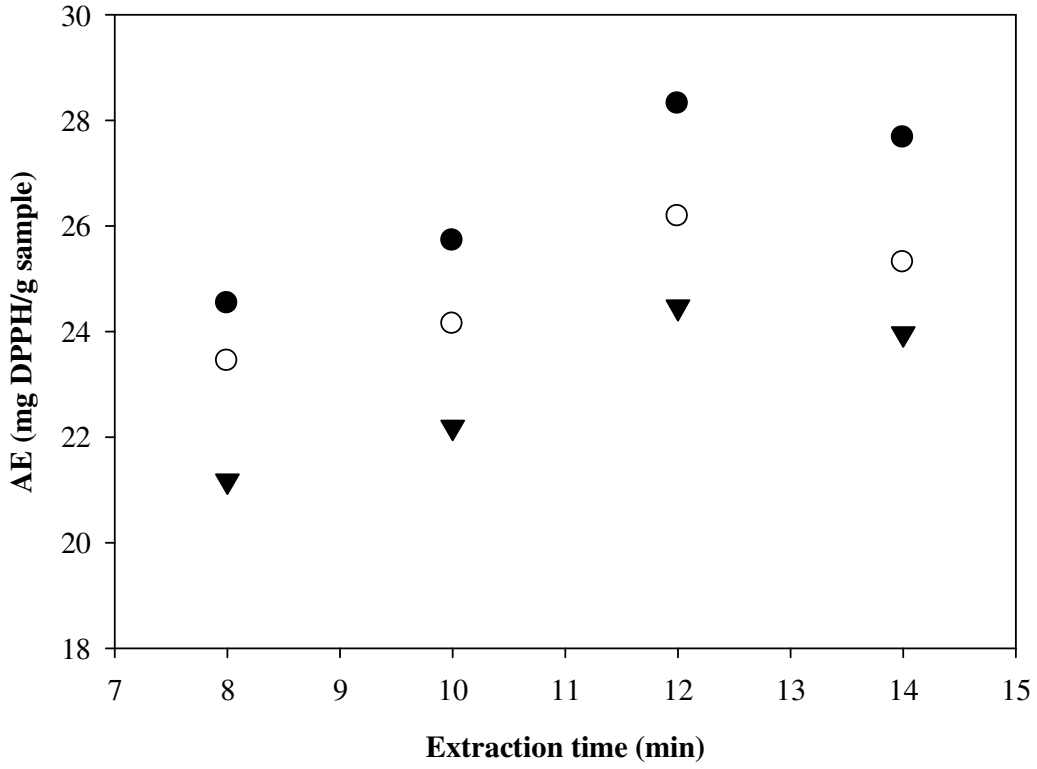


Figure 3.33. Effects of different solvent types on AE of sour cherry pomace extracts obtained using solvent to solid ratio of 20 ml/g during MAE at 700 W. (●): ethanol-water, (○): ethanol, (▼): water.

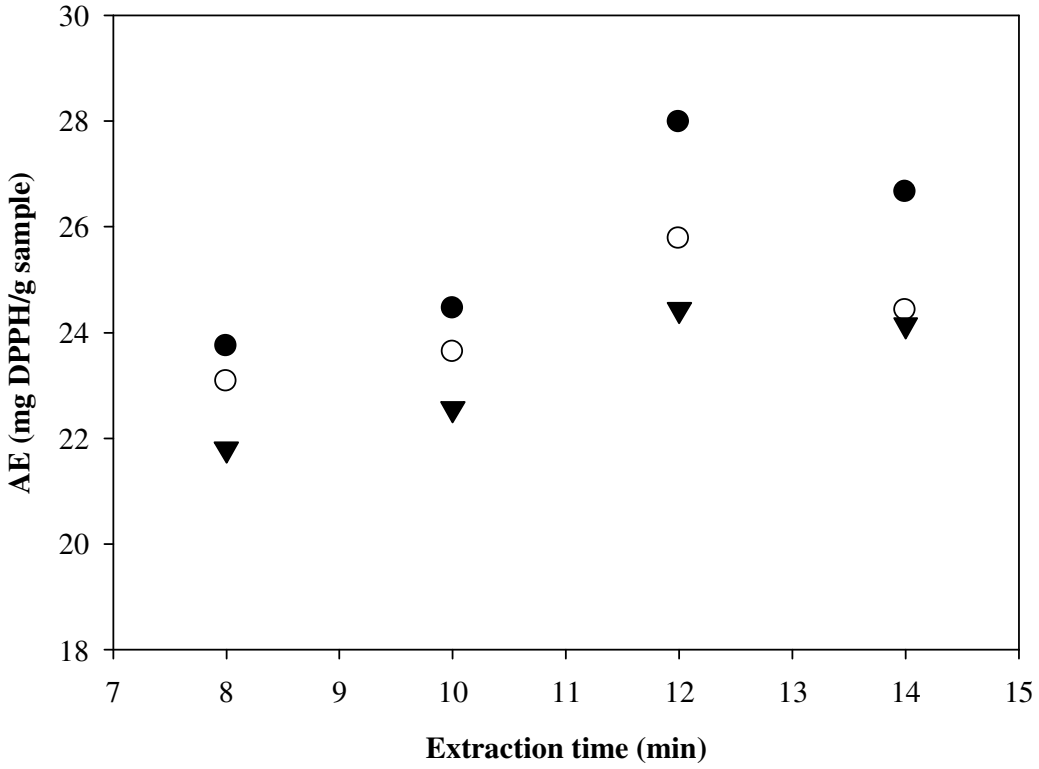


Figure 3.34. Effects of different solvent types on AE of sour cherry pomace extracts obtained using solvent to solid ratio of 30 ml/g during MAE at 700 W. (●): ethanol-water, (○): ethanol, (▼): water.

Similar to TPC results, Figure 3.35 demostrated that the highest AE was determined at a solvent to solid ratio of 20 ml/g when extraction solvent was chosen as ethanol-water .

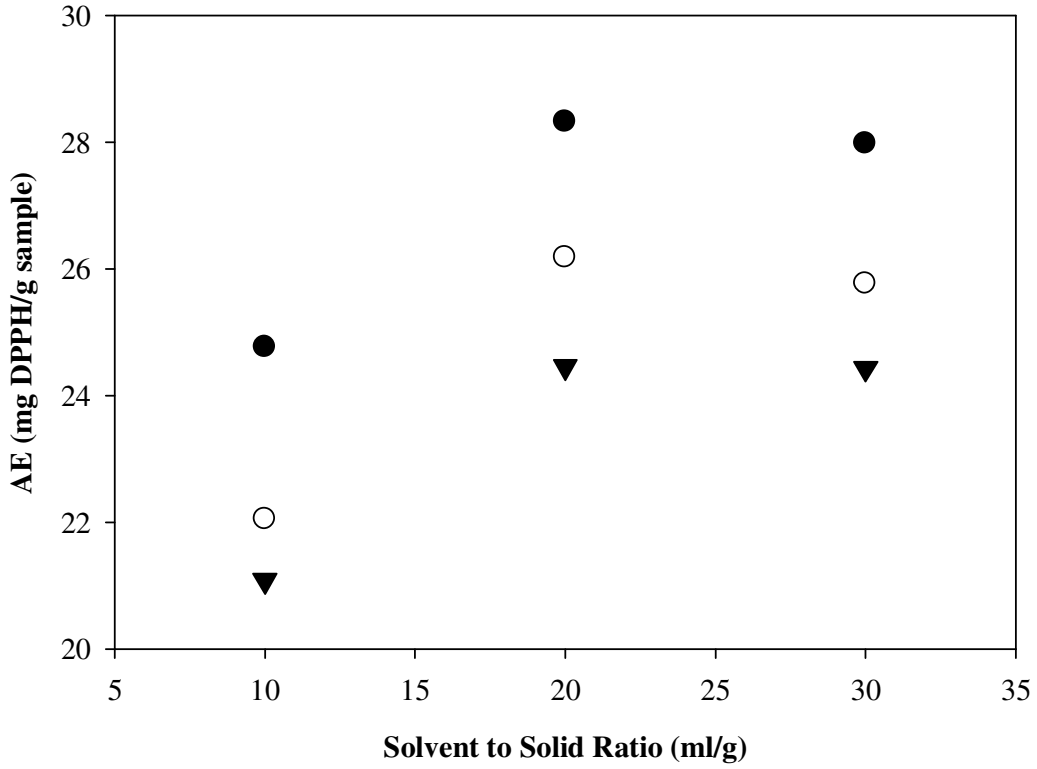


Figure 3.35. AE of sour cherry pomace extracts obtained using 700 W microwave power level, different solvent to solid ratios and optimum time (●): ethanol-water for 12 min, (○): ethanol for 12 min , and (▼):water for 12 min.

Figure 3.36 indicated that AE values of sour cherry pomace extracts obtained by conventional extraction were 24.74, 20.08 and 17.84 mg DPPH/g sample for ethanol-water, ethanol and water. AE was found as 28.32 mg DPPH/g sample at the optimum condition of MAE (700 W and solvent to solid ratio of 20 ml/g). As can be seen from these values, MAE improved AE significantly by 14.47% as compared to conventional extraction due to shorter extraction time as in the case of tomato

pomace extraction. The extraction method was determined as statistically significant ($p \geq 0.05$) on affecting AE of sour cherry pomace extracts.

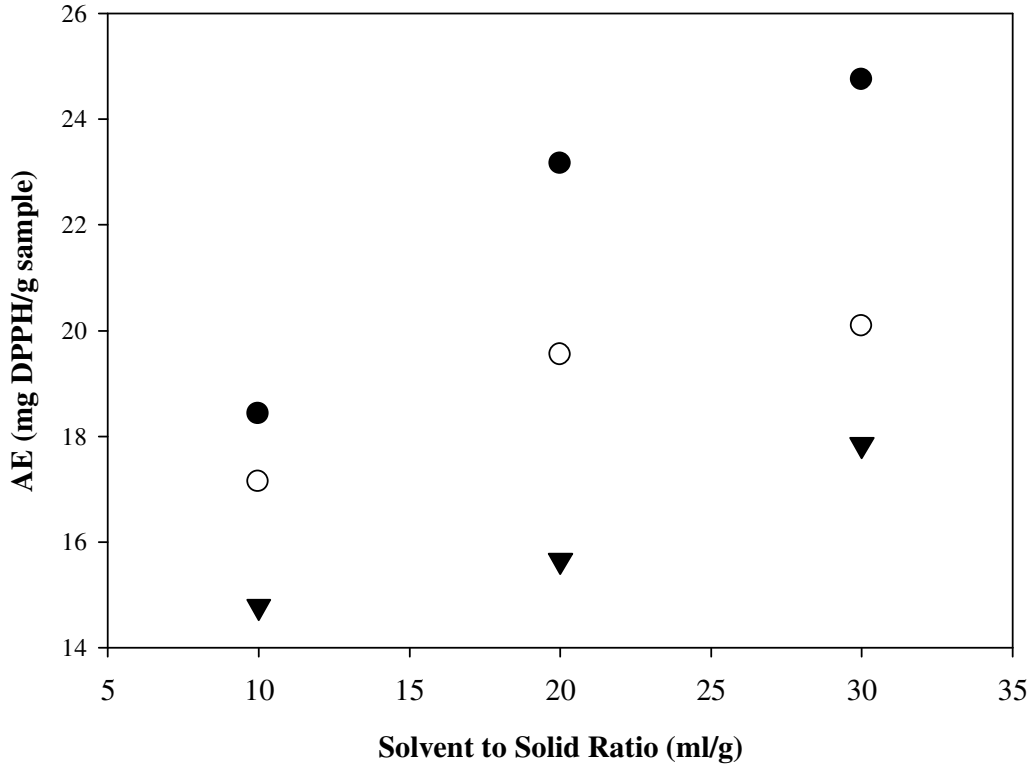


Figure 3.36. The effect of solvent to solid ratios and different solvents on AE of tomato pomace extract obtained with conventional extraction for 6 hours. (●): ethanol-water (○): ethanol, and (▼):water.

3.2.3. Concentration of Phenolic Acids of Sour Cherry Pomace Extracts Using HPLC

HPLC analysis was performed for the extracts obtained at optimum conditions. For MAE, the samples extracted using ethanol-water mixture, ethanol and water, 700 W, solvent to solid ratio of 20 ml/g and 12 min were used for HPLC analysis. For conventional extraction, the samples obtained at a solvent to solid ratio of 20 ml/g were used. The detected phenolic compounds were vannilic, epicatechin, syringic, gentisic and quercitrin. As can be seen from Table 3.2, extracts obtained by MAE

provided greater amount of these phenolic compounds as compared to the ones obtained in conventional extraction. As suggested in the evaluation of TPC and AE, the reason of obtaining higher concentrations of phenolic compounds could be related to the extraction time. In MAE, shorter extraction time might prevent the destruction of phenolic compounds, and therefore higher concentration of phenolic compounds can be obtained.

Table 3.2 Concentration of main phenolic acids in sour cherry pomace extracts (mg/g sample).

Extraction Method	Solvent Type	Vanillic	Epicatechin	Syringic	Gentisic	Quercitin
MAE	Ethanol-Water	0.0091	0.0219	0.01024	0.0128	0.0005
MAE	Ethanol	0.0047	0.0088	0.0038	0.0104	0.0001
MAE	Water	0.0017	0.0064	-	0.0062	-
Conventional	Ethanol-Water	0.0025	0.0119	0.0016	-	-
Conventional	Ethanol	0.0021	0.0041	0.0013	-	-
Conventional	Water	0.0018	0.0055	-	-	-

CONCLUSION AND RECOMMENDATIONS

In this study, extracts obtained by microwave assisted extraction were compared with the conventional extraction in terms of total phenolic content, antiradical efficiency and concentration of major phenolic compounds.

In MAE, solvent type, microwave power, solvent to solid ratio and extraction time were found to affect TPC and AE of tomato and sour cherry pomaces significantly. Higher TPC and AE were obtained in the presence of ethanol-water mixture as compared to other solvents in both extraction methods. The increase in power level increased TPC and AE of tomato and sour cherry pomace and shortened extraction time.

The optimum extraction time was found to change according to the type of pomace used in the extractions, type of the solvent and extraction method. The increase in solvent to solid ratio increased TPC and AE of the pomace extract when conventional extraction was used. However, there was an optimum solvent to solid ratio in the presence of MAE. Between the two samples, the highest TPC and AE were obtained from sour cherry pomace.

MAE improved AA and concentration of main phenolic acids significantly as compared to conventional extraction. This increase could be resulted from the shorter extraction time in MAE than conventional extraction. Thus, microwave assisted extraction can be considered as an alternative method in the extraction of phenolic compounds from these pomaces. It also decreases extraction time significantly which has an important role to obtain phenolic compounds showing low heat tolerance.

The interaction of ethanol-water mixture with the other organic compounds can be evaluated in the molecular level.

As a result, fruit pomaces can be utilized as a source of phenolic compounds and its extracts can be alternative natural antioxidants. The consumer demand of consuming food with natural ingredients can also be met with the utilization of these pomaces.

Further investigations can be focused on stability of these antioxidants. Other research might be related to the bioavailability of the extracted phenolic compounds and their health effects.

REFERENCES

Abushita, A. A., Daood, H. G., Biacs, P. A., 2000. Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. Journal of Agricultural and Food Chemistry, 48: 2075-2081.

Agarwal, A., Shen, H., Agarwal, S., Rao, A. V., 2001. Lycopene content of tomato products: Its stability, bioavailability and in vivo antioxidant properties. Journal of Medicinal Food, 4: 9-15.

Alonso-Salces, R. M., Korta, E., Barranco, A., Berrueta, L.A., Gallo, B., Vicente, F., 2001. Pressurized liquid extraction for the determination of polyphenols in apple. Journal of Chromatography A, 933: 37–43.

Anttonen, M. J., Karjalainen, R. O., 2005. Environmental and genetic variation of phenolic compounds in red raspberry. Journal of Food Composition and Analysis, 18: 759-769.

Aruoma, O. I., 2002. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutation Research, 523-524: 9-20.

Arts, I. C., van De Putte, B., Hollman, P.C., 2000. Catechin contents of foods commonly consumed in the Netherlands.2. Tea, wine, fruit juices, and chocolate milk. Journal of Agricultural and Food Chemistry., 48: 2848-2852.

Awad, M.A., deJager, A., van Westing, L.M., 2000. Flavonoid and chlorogenic acid levels in apple fruit:characterization of variation. Scientia Horticulturae, 83: 249–263.

Bagchi, K., Puri, S., 1998. Free radicals and antioxidants in health and disease. Eastern Mediterranean Health Journal, 4(2): 350-360.

Bagheri, H., Mohammadi, A., Salemi, A., 2004. On-line trace enrichment of phenolic compounds from water using a pyrrole-based polymer as the solid-phase extraction sorbent coupled with high-performance liquid chromatography. Analytica Chimica Acta, 513: 445–449.

Balasundram, N., Sundram, K., Samman, S., 2006. Phenolic compounds in plants and agri-industrial by products: Antioxidant activity, occurrence, and potential uses. Food Chemistry, 99(1): 191-203.

Barlow, M.S., 1990. Toxicological aspects of antioxidants used as food additives. In B.J.F. Hudson, Food Antioxidants (pp. 253). New York: Elsevier Science Publishers.

Beejmohun, V., Fliniaux, O., Grand, E., Lamblin, F., Bensaddek, L., Christen, P., Kovensky, J., Fliniaux, M. A., Mesnard, F., 2007. Microwave-assisted extraction of the main phenolic compounds in flaxseed. Phytochemical Analysis, 18: 275-282.

Bengoechea, M. L., Sancho, A. I., Bartolome, B., Estrella, I., Gomez-Cordoves, C., Hernandez, M. T., 1997. Phenolic composition of industrially manufactured purees and concentrates from peach and apple fruits. Journal of Agricultural and Food Chemistry. 45: 4071–4075.

Bertelli, A., Bertelli, A.A.E., Gozzini, A., Giovannini, L., 1998. Plasma and tissue resveratrol concentrations and pharmacological activity. Drugs Under Experimental and Clinical Research, 24:133-138.

Bilyk, A., Cooper, P. L., Sapers, G. M.,1984. Varietal differences in distribution of quercetin and kaempferol in onion (*Allium cepa* L.) tissue. Journal of Agricultural and Food Chemistry, 32: 274 - 276.

Bocco, A., Cuvelier, M. E., Richard, H., Berset, C., 1998. Antioxidant activity and phenolic composition of citrus peel and seed extracts. Journal of Agricultural and Food Chemistry, 46: 2123-2129.

Bravo, L., 1998. Polyphenols: chemistry, dietary sources, and metabolism, and nutritional significance. Nutrition Reviews, 56: 317-333.

Burg, P., Fraile, P., 1995. Vitamin-c destruction during the cookimg of a potato dish. Food Science and Technology, 28: 506- 514.

Cacace, J. E., Mazza, G., 2003. Mass transfer process during extraction of phenolic compounds from milled berries. Journal of Food Engineering, 59:379-389.

Carabies-Martinez, R., Rodriguez-Gonzalo, E., Revilla-Ruiz, P., Hernandez-Mendez, J., 2005. Pressurized liquid extraction in the analysis of food and biological samples. Journal of Chromatography A, 1089: 1-17.

Carotenuto, A., Fattorusso, E., Lanzotti, V., Magno, S., De Feo, V., Cicala, C., 1997. The flavonoids of Allium neapolitanum. Phytochemistry. 44: 949 - 957.

Cevallos-Casals, B. A., Byrne, D., Okie, W. R., Cisneros-Zevallos, L., 2006. Selecting new peach and plum genotypes rich in phenol compounds and enhanced functional properties. Journal of Food Chemistry, 273-280.

Chandra, A., Nair, M.G., Lezzoni, A., 1992. Evaluation and characterization of the anthocyanin pigments in tart cherries (Prunus cerasus L.) Journal of Agricultural and Food Chemistry, 40: 967-969.

Chandra, A., Rana, J., Li, Y., 2001. Seperation ,identification, quantification and Method Validation of Anthocyanins in Botanical Supplement Raw Materials by HPLC and HPLC-MS.

Chaovanalikit, A., Wrolstad, R. E., 2004. Total Anthocyanins and Total Phenolics of Fresh and Processed Cherries and Their Antioxidant Properties. Journal of Food Science, 69: 67-72.

Chang, S., Tan, C., Frankel, E. N., Barrett, D. M., 2000. Low-density lipoprotein antioxidant activity of phenolic compounds and polyphenoloxidase activity in selected clingstone peach cultivars. Journal of Agricultural and Food Chemistry, 48: 147–151.

Chang, C. H., Lin, H. Y., Chang, C. Y., Liu, Y. C, 2006. Comparisons on the antioxidant properties of fresh, freeze-dried and hot-air-dried tomatoes. Journal of Food Enginnering, 77: 478- 485.

Chen, L., Jin, H., Ding, L., Zhang, H., Li, J., Qu, C., Zhang, H., 2008. Dynamic microwave-assisted extraction of flavonoids from Herba Epidemii. Separation and Purification Technology, 59: 50-57.

Chen, R. Y., Wu, J. J., Tsai, M. J., Liu, M. S., 2000. Effect of storage and thermal treatment on the antioxidant activity of tomato fruits. Journal of the Chinese Agricultural Chemical Society, 38: 353-360.

Chen, H., Zuo, Y., Deng, Y., 2001. Seperation and determination of flavonoids and other phenolic compounds in cranberry juice by high-performance liquid chromatography. Journal of Chromatography A, 913: 387 – 395.

Clifford, M. N., 2000. Anthocyanins-nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture, 80: 1063-1072.

Criado, M. R., Torre, S. P., Pereiro, I. R., Torrijos, R. C., 2004. Optimization of a microwave-assisted derivatization-extraction procedure for the determination of chlorophenols in ash samples. Journal of Chromatography A, 1024: 155-163.

Devasagayam, T. P. A., Tilak, J. C., Boloor, K. K., Sane, K. S., Ghaskadbi, S. S., Lele, R. D., 2004. Free radicals and antioxidants in human health: Current status and future aspects. The Journal of the Association of Physicians of India, 794-804.

Dewick, P. M., 1993. The biosynthesis of shikimate metabolites. Natural Products Reports. 10: 233-263.

Dewick, P. M., 2002. Medicinal Natural Products: A Biosynthetic Approach. John Wiley & Sons, Ltd.

Dick, A. J., Redden, P. R., Demarco, A. C., Lidster, P. D., Grindley, T. B., 1987. Flavonoid glycosides of Spartan apple peel. Journal of Agricultural and Food Chemistry, 35: 529-531.

Duvernay, W. H., Assad, J. M., Sabliov, C. M., Lima, M., Xu, Z., 2005. Microwave extraction of antioxidant components from rice bran. The Official Journal of ISPE, 1-5.

Eskillson, C. S., Bjorklund, E., 2000. Anaytical scale microwave-assisted extraction. Journal of Chromatography A, 902: 227-250.

Filgueiros, A.V., Capelo, J. L., Lavilla, I., Bendicho, C., 2000. Comparison of ultrasound-assisted extraction and microwave-assisted digestion for determination of magnesium, manganese and zinc in plant samples by flame atomic absorption spectrometry. Talanta, 53:433-441.

Fiorentino, G., Spaccini, R., Piccolo, A. 2006. Separation of molecular constituents from a humic acid by solid-phase extraction following a transesterification reaction. Talanta, 68 (4): 1135-1142.

Fu, C., Tian, H., Li, Q., Cai, T., Du, W., 2006. Ultrasound-assisted extraction of xyloglucan from apple pomace. Ultrasonics Sonochemistry, 13: 511-516.

Gahler, S., Otto, K., Bohm, V., 2003. Alterations of vitamin c, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products. Journal of Agricultural and Food Chemistry, 51: 7962- 7968.

Gao, L., Mazza, G., 1995. Characterization, quantitation and distribution of anthocyanins and colorless phenolics in sweet cherries. Journal of Agricultural and Food Chemistry, 43: 343-346.

Gao, M., Song, M., Lin, C., 2006. Dynamic microwave assisted extraction of flavonoids from Saussurea Medusa Maxim.Cultured Cells. Biochemical Engineering Journal, 332: 79-93.

Garcia-Viguera, C., Bridle, P., Ferreres, F., Tomás-Barberán, F. A., 1994. Influence of variety, maturity and processing on phenolic compounds of apricot

juices and jams. Zeitschrift fur Lebensmittel-Untersuchung und Forschung, 199: 433-436.

Garcia, Y. D., Valles, B. S., Lobo, A. P., 2009. Phenolic and antioxidant composition of by-products from the cider industry: Apple pomace. Food Chemistry, 117: 731-738.

George, B., Kaur, C., Khudiya, D. S., Kapoor, H. C., 2004. Antioxidants in tomato (Lycopersium esculentum) as a function of genotype. Food Chemistry, 84: 45-51.

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Kader, A. A., 2002. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. Journal of Agricultural and Food Chemistry, 50: 4976–4982.

Giuntini, D., Graziani, G., Lercari, B., Fogliano, V., Soldatini, G. F., Ranieri, A., 2005. Changes in carotenoid and ascorbic acid contents in fruits of different tomato genotypes related to the depletion of UV-B radiation. Journal of Agriculture and Food Chemistry, 53: 3174-3181.

Goli, A. H., Bargezar, M., Sahari, M. A., 2005. Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. Food Chemistry, 92: 521-525.

Goncalves, B., Landbo, A. K., Knudsen, D., Silva, A. P., Moutinho-Pereira, J., Roja, E., Meyer, A. S., 2004. Effect of ripeness and postharvest storage on the phenolic profiles of cherries (Prunus avium L.). Journal of Agricultural and Food Chemistry, 52: 523-530.

Gordon, M. H., 1990. The mechanism of antioxidant action *in vitro*. In: Food

antioxidants. Hudson, B.J.F. (ed). Elsevier Applied Science, London. 1–18.

Gorinstein, S., Martin-Belloso, O., Park Y. S., Haruenkit, R., Lojek, A., Ciz, M., Caspi, A.,Libman, I., Trakhtenberg, S., 2001. Comparison of some biochemical characteristics of different citrus fruits. Food Chemistry, 74: 309–315.

Gorinstein, S., Martin-Belloso, O., Lojek, A., Ciz, M., Soliva-Fortuny, R., Park, Y.S., Caspi, A., Libman, I., Trakhtenberg, S., 2002. Comparative content of some phtochemicals in Spanish apples, peaches and pears Journal of The Science of Food and Agriculture, 82: 1166-1170.

Gorinstein, S., Cvikrová, M., Machackova, I., Harvenkit, R., Park, Y. S., Jung, S-T., Yamamoto, K., Ayala, A. L. M., Katrich, E., Trakhtenberg, S., 2004. Characterization of antioxidant compounds in Jaffa sweeties and white grape fruits. Journal of Food Chemistry, 84: 503 - 510.

Grigonis, D., Venskutonis, P. R., Sivik, B., Sandahl, M., Eskilsson, C. S., 2005. Comparison of different extraction techniques for isolation of antioxidants from sweet grass (*Hierochloë odorata*). Journal of Supercritical Fluids, 33: 223–233.

Guo, Z., Fan, G., Duan, Y., Qin, C., Wen, M., 2001. Microwave-assisted extraction of effective constituents from a Chinese herbal medicine Radix puerariae. Analytica Chimica Acta, 436: 41-47.

Halliwell, B., 1994. Free radicals, antioxidants and human disease: curiosity, cause or consequence. The Lancet, 344, 721-724.

Halliwell, B., Murcia, M. A., Chirico, S., Aruoma, O. I., 1995. Free radicals and antioxidants in food and *in vivo*: what they do and how they work. Critical Reviews in Food Science and Nutrition, 35: 7-20.

Harborne, J.B., 1989. General procedures and measurement of total phenolics. In J.B. Harborne (Ed.) Methods in plant biochemistry, 1: 1-28.

Harborne, J. B., Baxter, H., Moss, G.P., 1999. Phytochemical dictionary: Handbook of bioactive compounds from plants (2nd ed.). London: Taylor & Francis.

Harborne, J. B., Williams, C. A., 2000. Advances in flavonoid research since 1992. Phytochemistry, 55: 481 - 504.

Hasbay, I., Yener, E., Bayindirli, A., 2008. Extraction of total phenolics of sour cherry pomace by high pressure solvent and subcritical fluid and determination of antioxidant activities of the extracts. Separation Science and Technology, 43: 1091-1110.

Hayat, K., Hussain, S., Abbas, S., Farooq, U., Ding, B., Xia, S., Jia, C., Zhang, X., Xia, W., 2009. Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity in vitro. Seperation and Purification Technology, 70: 63-70.

Heinonen, I. M., Lehtonen, P.J., Hopia, A. I., 1998. Antioxidant activity of berry and fruit wines and liquors. Journal of Agricultural and Food Chemistry, 46: 25-31.

Henning, W., Herrmann, K., 1980a. Flavonol glycosides of plums of the species *Prunus domestica L.* and *Prunus salicina* Lindley.12. Phenolic content of fruits. Zeitschrift fur Lebensmittel-Untersuchung und Forsuchung, 171: 111-118.

Henning, W., Herrmann, K., 1980b. Flavonol glycosides of apricots (*Prunus armeniaca* L.) and (*Prunus persica* Batsch).13. Phenolic content of fruits. Zeitschrift fur Lebensmittel- Untersuchung und Forsuchung, 171: 183-188.

Herrera, M. C., Luque de Castro, M. D., 2005. Ultrasound-assisted extraction of phenolic compounds from strawberries prior to liquid chromatographic separation and photodiode array ultraviolet detection. Journal of Chromatography A, 1100: 1–7.

Hertog, M. G., Feskens, E. J., Hollman, P. C., Katan, M. B., Kromhout, D., 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zupthen Elderly Study. Lancet, 342: 1007-1011.

Hoff, J. F., Singleton, K. I. A., 1977. A method for determination of tannin in foods by means of immobilized enzymes. Journal of Food Science, 42: 15666.

Hollman P.C.H., Hertog M.G.L., Katan M.B, 1996. Role of dietary flavonoids in protection against cancer and coronary heart disease. Biochemical Society Transactions, 24:785-789.

Hong, V., Wrolstad, R. E., 1986. Cranberry juice composition. Journal of the Association of official Analytical Chemists, 69: 199-207.

Howard, L. R., Pandjaitan, N., Morelock, T., Gil, M. I., 2002. Antioxidant capacity and phenolic content of spinach as affected by genetics and growing season. Journal of Agricultural and Food Chemistry, 50: 5891 - 5896.

Huang, M.T., Ho, C. T., Lee, C. Y., 1992. Phenolic compounds in food and their effects on health II. ACS Symposium Series 507. American Chemical Society, Washington, DC.

Huie, C. W., 2002. A review of modern sample preparation techniques for the extraction and analysis of medicinal plants. Analytical and Bioanalytical Chemistry, 373: 23-30.

Hung, H. C., Joshipura, K. J., Jiang, R., Hu, F. B., Hunter, D., Smith-Warner, S. A., Colditz, G. A., Rosner, B., Spiegelman, D., Willett, W. C., 2004. Fruit and vegetable intake and risk of major chronic disease. Journal of National Cancer Institute, 96: 1577-1584.

Ju, Z. Y., Howard, L. R., 2003. Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin. Journal of Agricultural and Food Chemistry, 51: 5207–5213.

Kammerer, D., Claus, A., Carle, Reinhold, Schieber, A., 2004. Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. Journal of Agriculture and Food Chemistry, 52: 4360-4367.

Kanner, J., Frankel, E., Granit, R., German, B., Kinsella, J. E., 1994. Natural antioxidants in grapes and wines. Journal of Agricultural and Food Chemistry, 42: 64 – 69.

Karadeniz, F., Burdurlu, H. S., Koca, N., Soyer, Y., 2005. Antioxidant activity of selected fruits and vegetables grown in Turkey. Turkish Journal of Agriculture and Forestry, 29: 297– 303.

Khokhar, S., Magnusdottir, S. G. M., 2002. Total phenol, catechin and caffeine contents of teas commonly consumed in the United Kingdom. Journal of Agricultural and Food Chemistry, 50:565- 570.

Kim, D. O., Heo, H. J., Kim, Y. J., Yang, H. S., Lee, C. Y., 2005. Sweet and sour cherry phenolics and their protective effects on neuronal cells. Journal of Agricultural and Food Chemistry, 53: 9921-9927.

Koca, I., Karadeniz, B., 2009. Antioxidant properties of blackberry and blueberry fruits grown in the Black Sea Region of Turkey. Scientia Horticulturae, 121: 447-450.

Kroon, P.A., Williams, G., 1999. Hydroxycinnamates in plants and food: current and future perspectives. Journal of the Science of Food and Agriculture, 79: 355-361.

Kwon, J., Lee, G., Belanger, J. M. R., Pare, J. R. J., 2003. Effect of ethanol concentration on the efficiency of extraction of ginseng saponins when using a microwave assisted process. Journal of Food Science and Technology, 38: 615-622.

Laroze, L., Zuniga-Hansen, M. E., 2007. Phenolic antioxidant extraction from selected agroindustrial residual sources. Journal of Biotechnology, 131: 188-195.

Larrauri, J. A., Ruperez, P., Calixto, F. S., 1996. Antioxidant activity of wine pomace. Journal of Enology and Viticulture, 47(4): 369-372.

Lasheras, C., Fernandez, S., Patterson, A. M., 2000. Mediterranean diet and age with respect to overall survival in institutionalized, non-smoking elderly people. American Journal of Clinical Nutrition, 71: 987- 992.

Lathrop, P. J., Leung, H. K., 1980. Rates of ascorbic-acid degradation during thermal-processing of canned peas. Journal of Food Science, 45: 152-153.

Lee, J., Wrolstad, R. E., 2004. Extraction of anthocyanins and polyphenolics from blueberry processing waste. Journal of Food Science, 69: 564 – 573.

Leantowicz, H., Gorinstein, S., Lojek, A., Leantowicz, M., Ciz, M., Soliva-Fortuny, R., Park, Y. S., Jung, S. T., Trakhtenberg, S., Martin-Belloso, O., 2002. Journal of Nutritional Biochemistry, 13: 603- 610.

Letellier, M., Budzinski, H., 1999. Microwave assisted extraction of organic compounds. Analusis, 27: 259-271.

Letellier, M., Budzinski, H., Charrier, L., Capes, S., Dorthe, A. M., 1999. Optimization by factorial design of focused microwave assisted extraction of polycyclic aromatic hydrocarbons from marine sediment. Journal of Analytical Chemistry, 364: 228-237.

Li, H., Chen, B., Zhang, Z., Yao, S., 2004. Focused microwave assisted solvent extraction and HPLC determination of effective constituents in *Eucommia ulmodies Oliv.*, Talanta, 63: 659-665.

Li,Y., Guo, C., Yang, J., Wei, J., Xu, J., Cheng, S., 2005. Evaluation of antioxidant properties of pomegranate peel extraction comparison with pomegranate pulp extract. Food Chemistry, 96: 254 - 260.

Lin, J.-H., Chiou, Y.-N. and Lin, Y.-L., 2002. Phenolic glycosides from Viscum angulatum. Journal of Natural Products, 65: 638 - 640.

Liu, R. H., 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. Journal of Nutrition, 134: 3479-3485.

Lommen, A., Godejohann, M., Venema, D. P., Hollman, P. C. H., Spraul, M., 2000. Application of directly coupled HPLC-NMR-MS to the identification and confirmation of quercetin glycosides and phloretin glycosides in apple peel. Analytical Chemistry, 72: 1793–1797.

Lu, Y., Foo, L. Y., 1998. Constitution of some chemical components of apple seed. Food Chemistry, 61: 29-33.

Lucchesi, M. E., Smadja, J., Bradshaw, S., 2007. Solvent free microwave extraction of Elletaria cardomomum L.: A multivariate study of new technique for the extraction of essential oil. Journal of Food Engineering, 79 (3): 1079-1086.

Luque- Garcia, J. L., Luque de Castro, M. D., 2003. Ultrasound: a powerful tool for leaching. Trends in Anaytical Chemistry, 22: 41-47.

Manach, C., Scalbert, A., Morand, C., Remesy, C., Jimenez, L., 2004. Polyphenols: food sources and bioavailability. American Journal of Clinical Nutrition, 79: 727-747.

Mandal, V., Mohan, Y., Hemalatha, S., 2007. Microwave assisted extraction-an innovative and promising extraction toll for medicinal plant research. Pharmacognosy Reviews, 1: 7-18.

Mann, J., 1987. Secondary metabolism. Oxford University Press. Toronto, ON.

Manzocco, L., Anese, M., Nicoli, M., 1998. Antioxidant properties of tea extracts as affected by processing. Lebensmittel Wissenschaft und Technologie, 31: 694-698.

Mazza, G., Fukumoto, l., Delaquis, P., Girard, B., Ewert, B., 1999. Anthocyanins, phenolics, and color of Cobernet Franc, Merlot, and Pinot Noir wines from British Colombia. Journal of Agricultural and Food Chemistry, 47: 4009- 4017.

Meyer, A. S., Donovan, J. L., Pearson, D. A., Waterhouse, A. L., Frankel, E. N., 1998. Fruit hydrocinnamic acids inhibit human low-density lipoprotein oxidation in vitro. Journal of Agricultural and Food Chemistry, 46: 1783-1787.

Mirdehghan, S. H., Rahemi, M., 2007. Seasonal changes of mineral nutrients and phenolics in pomegranate (*Punica granatum* L.) fruit. Scientia Horticulturea, 111: 120-127.

Mansouri, A., Embarek, G., Kokkalou, E., Kefalas, P., 2005. Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoneix dactylifera*). Journal of Food Chemistry, 89: 411-420.

Mudgett, R. E., 1995. Electrical properties of foods. In Rao, M. A., Rizvi, S.S.H., Eds. Enginnering properties of foods 2nd edn. Markel Dekker, New York.

Murcia, M. A., Lopez-Ayerra, B., Martinez- Tome, M., Vera, A. M., Garcia-Carmona, F., 2000. Evaluation of ascorbic acid and peroxidase during industrial processing of broccoli. Journal of the Science of Food and Agriculture, 80: 1882-1886.

Noguchi, N., Niki, E., 1998. Chemistry of active oxygen species and antioxidants. In: Antioxidant status, diet, nutrition, and health. Papas, A.M. (ed). CRC Press, Boca Raton, FL. 3–20.

Norbeak, R. and Kondo, T., 1999. Flavonol glycosides from flowers of Crocus speciosus and C. antalyensis. Phytochemistry, 51: 1113 - 1119.

Obied, H. K., Allen, M. S., Bedgood, D. R., Prenzler, P. D., Robards, K., Stockmann, R., 2005. Bioactivity and analysis of biophenols recovered from olive mill waste. Journal of Agricultural and Food Chemistry, 53: 823–837.

Palma, M., Taylor, L. T., 1999. Extraction of polyphenolic compounds from grape seeds with near critical carbon dioxide. Journal of Chromatography A, 849: 117-124.

Palma, M., Pineiro, Z., Barroso, C.G., 2002. In-line pressurized-fluid extractionsolid-phase extraction for determining phenolic compounds in grapes. Journal of Chromatography A, 968: 1-6.

Pan, X., Niu, G., Liu, H., 2001. Microwave assisted extraction of tanshinones from Salvia miltiorrhiza bunge with analysis by high performance liquid chromatography**.** Journal of Chromatography A, 922: 371-375.

Pan, X., Guaguang, N., Liu, H., 2003. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. Chemical Engineering and Processing , 42: 129-133.

Papagiannopoulos, M., Zimmermann, B., Mellenthin, A., Krappe, M., Maio, G., Galensa, R., 2002. Online coupling of pressurized liquid extraction, solid-phase extraction and high-performance liquid chromatography for automated analysis of proanthocyanidins in malt. Journal of Chromatography A, 958: 9–16.

Parr, A. J., Bolwell, G. P., 2000. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. Journal of the Science of Food and Agriculture, 80: 985-1012.

Pathirana, C., Shahidi, F., 2005. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chemistry, 93(1): 47-56.

Picinelli, A., Suarez, B., Mangas, J. J., 1997. Analysis of polyphenols in apple products. Zeitschrift fur Lebensmittel-Untersuchung and Forschung A, 204: 48-51.

Pierpoint, W.S., 1986. Flavonoids in the human diet. Progress in Clinical Biological Research, 213:125-140.

Pietta, P.G., 2000. Flavonoids as antioxidants. Journal of Natural Products, 63:1035-1042.

Pokorny, J., 2007. Are natural antioxidants better - and safer - than synthetic antioxidants? European Journal of Lipid Science and Technology, 109: 629-642.

Poyrazoglu, E., Gokmen, V., Artik, N., 2002. Organic acids and phenolic compounds in pomegranates (*Punica granatum L.)* grown in Turkey. Journal of Food Composition and Analysis, 15: 567-575.

Price, K. R., Prosser, T., Richetin, A. M. F., Rhodes, M. J. C., 1999. A comparison of the flavonol content and composition in dessert, cooking and cider-makingapples; distribution within the fruit and effect of juicing. Food Chemistry, 66: 489–494.

Proestos, C., Komaitis, M., 2008. Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. Food Science and Technology, 41: 652-659.

Punchard, N. A., Kelly, F.J., 1996. Free radicals: A practical approach. New York: Oxford University Press.

Ranalli, A., Lucera, L., Contento, S., 2003. Antioxidizing potency of phenol compounds in olive mill waste water. Journal of Agricultural and Food Chemistry, 51: 7636–7641.

Rao, M. A., Lee, C. Y., Katz, J., Cooley, H. J., 1981. A kinetic study of the loss of vitamin-c, color, and firmness during thermal processing of canned peas. Journal of Food Science, 46: 636- 637.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., Glover, W., 1999. Phenolic compounds and their role in oxidative processes in fruits. Food Chemistry, 66: 401-436.

Romero, M., Roman, D., Carretero, A., Gutierrez, A., 2007. Anaytical determination of antioxidants in tomato: Typical components of the Mediterranean diet. Journal of Separation Science, 30: 452-461.

Rommel, A., Wrolstad, R. E., Heatherbell, D. A., 1992. Blackberry juices and wine: processing and storage effects on anthocyanin compostion, color and appearance. Journal of Food Science, 57: 385-391.

Rommel, A., Wrolstad, R. E., 1993. Composition of flavonols in red raspberry juice as influenced by cultivar, processing, and environmental factors. Journal of Agricultural and Food Chemistry, 41: 1941-1950.

Rop, O., Jurikova, T., Mlcek, J., Kramarova, D., Sengee, Z., 2009. Antioxidant activity and selected nutritional values of plums (*Prunus domestica* L.) typical of the white Carpathian Mountains. Scientia Horticulturea, 12: 545-549.

Rostagno, A. M., Palma, M., Barroso, C. G., 2005. Solid phase extraction of soy isoflavones. Journal of Chromatography A, 1076: 110-117.

Rostagno, M. A., Palma, M., Barroso, C. G., 2007. Microwave assisted extraction of soy isoflavones. Analytica Chimica Acta, 588: 274-282.

Saitoh, T., Nakayama, Y., Hiraide, M., 2002. Concentration of chlorophenols in water with sodium dodecylsulfate-ɣ-alumina admicelles for high-performance liquid chromatographic analysis. Journal of Chromatography A, 972: 205–209.

Sánchez-Moreno, C., Cao, G., Ou, B., Prior, R. L., 2003. Anthocyanin and proanthocyanidins content in selected white and red wines. Oxygen radical absorbance capacity comparison with nontraditional wines obtained from highblush blueberry. Journal of Agricultural and Food Chemistry, 51: 4889-4896.

Santana, C.M., Ferrera, Z.S., Padrón, M.E.T., Rodríguez, J.J.S., 2009. Methodologies for the extraction of phenolic compounds from environmental samples: New approaches. Molecules, 14: 298-320.

Santos- Buelga, C., Scalbert, A., 2000. Proanthocyanidins and tannin-like compounds: nature, accurrence, dietary intake and effects on nutrition and health. Journal of the Science of Food and Agriculture, 80: 1094-1117.

Schieber, A., Hilt, P., Streker, P., Endreß, H.-U., Rentschler, C., Carle, R., 2003. A new process for the combined recovery of pectin and phenolic compounds from apple pomace. Innovative Food Science and Emerging Technologies, 4: 99–107.

Schuster, B., Hemann, K., 1985. Hydroxybenzoic and hydroxycinnamic acid derivatives in soft fruits. Phytochemistry, 24: 2761.

Seeram, N. P., Nair, M. G., 2002. Inhibition of lipid oxidation and structure-activity-related studies of dietary constituents anthocyanins, anthocyanidins and catechins. Journal of Agricultural Food Chemistry, 50: 5308-5312.

Singleton, V. L., Rossi, J. A., 1965. Colorimetry of total phenolics with phosphomolibdic phosphotungstic acid reagent. American Journal of Enology and Viticulture, 16: 144-158.

Shahidi, F., Naczk, M., 1995. Food Phenolics: Sources, chemistry, effects, applications. Lancaster PA: Technomic Publishing Company Inc.

Shahidi, F., Naczk, M., 2004. Extraction and analysis of phenolics in food. Journal of Chromatography A, 1054, 95-111.

Shi, J., Yu, J., Pohorly, J. E., Kakuda, Y., 2003. Polyphenolics in grapes seeds. Biochemistry and Functionality. Journal of Medicinal Food, 6: 291-299.

Shrikhande, A. J., 2000. Wine by-products with health benefits. Food Research International, 33: 469–474.

Singleton, V.L., Rossi, J.A. Jr., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid Reagents, American Journal of Enology and Viticulture, 16: 144-158.

Someya, S., Yoshiki, Y., Okubo, K., 2002. Antioxidant compounds from bananas (Musa cavendish). Food Chemistry, 79: 351–354.

Stahl, W., Sies, H., 1992. Uptake of lycopene and its geometrical-isomers is greater from heat-processed than from unprocessed tomato juice in humans. Journal of Nutrition, 122: 2161-2166.

Sterbova, D., Matejicek, D., Vlcek, J., Kuban, V., 2004. Combined microwave-assisted isolation and solid-phase purufucation procedures prior to the chromatogrsphic determination of phenolic compounds in plant materials. Analytica Chimica Acta, 513: 435-444.

Stewart, A. J., Bozonnet, S., Mullen, W., Jenkins, G. I., Lean, M. E. J., Crozier, A., 2000. Occurrence of flavonols in tomatoes and tomato-based products. Journal of Agricultural and Food Chemistry, 48: 2663–2669.

Su, M. S., Silva, J. L., 2006. Antioxidant activity, anthocyanins, and phenolics of rabbiteye blueberry (*Vaccinium ashei*) by- products as affected by fermentation. Journal of Food Chemistry, 97: 447-451.

Sun, J., Chu, Y. F., Wu, X., Liu, R.H., 2002. Antioxidant and antiproliferative activities of common fruits. Journal of Agricultural and Food Chemistry, 50: 7449-7454.

Talebi, M., Ghassempour, Z., Talebpour, A., Dolatyari, L., 2004. Optimization of the extraction of paclitaxel from *Taxus baccata* L. by the use of microwave energy. Journal of Separation Science, 27: 1130-1136.

Talcott, S. T., Howard, L. R. and Brenes, C. H., 1999. Chemical and sensory quality of processed carrot puree as influenced by stress-induced phenolic compounds. Journal of Agricultural and Food Chemistry, 47: 1362-1366.

Tanaka, T., Takahashi, R., Kouno, I., Nonaka, G., 1994. Chemical evidence for the deastringency (insolubilization of tannins) of persimmon fruit. Journal of Chemical Society, 1: 3013-3022.

Tanrioven, D., Eksi, A., 2005. Phenolic compounds in pear juice from different cultivars. Journal of Food Chemistry, 93: 89-93.

Temple, N. J., 2000. Antioxidants and disease: more questions than answers. Nutrition Research, 20: 449-459.

Thompson, L. U., 1994. Antioxidant and hormone-mediated health benefits of whole grains. Critical Review Food Science and Nutrition, 34: 473-497.

Teuber, H., Herrmann, K., 1978. Flavonolglykoside der Apfel (*Malus silvestris* Mill.). Zeitschrift fur Lebensmittel-Untersuchung und-Forschung, 166: 80–84.

Thimothe, J., Bonsi, I. A., Padilla- Zakour, O. I., Koo, H., 2007. Chemical characterization of red wine grape (*Vitis vinifera* and *Vitis* interspecific hybrids) and pomace phenolic extracts and their biological activity against *Streptococcus* mutans. Journal of Agriculture and Food Chemistry, 55: 10200-10207.

Tomás- Barberán, F.A., Clifford, M.N., 2000. Dietary hydoxybenzoic acid derivatives- nature, occurrence, and dietary burden. Journal of the Science of Food and Agriculture, 80: 1024-1032.

Toor, R.K., Savage, G.P., 2005. Antioxidant activity in different fractions of tomatoes. Food Research International, 38: 487-494.

Torres, J. L., Bobet, R., 2001. New flavanol derivatives from grape (Vitisvinifera) byprodcts:antioxidant aminoethylthio-flavan-3-ol conjugates from a polymeric waste fraction used as a source of flavanols. Journal of Agricultural and Food Chemistry, 49: 4627–4634.

Usenik, V., Fabcic, J., Stampar, F., 2008. Sugars, organic acids, phenolic composition and antioxidant activity of sour cherry (*Prunus avium* L.). Food Chemistry, 107: 185-192.

Valverde, I., Periago, M. J., Provan, G., Chesson, A., 2002. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). Journal of the Science of Food and Agriculture, 82: 323-330.

Velioglu, Y.S., Mazza, G., Gao, L., Oomah, B. D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry, 46: 4113-4117.

Virot, M., Tomao, V., Bourvellec, C. L., Renard, C. M. C. G., Chemat, F., 2009. Towards the industrial production of antioxidants from food processing by-products with ultrasound-assisted extraction. Ultrasonics Sonochemistry, 1-9.

Visioli and Galli, 2003. Olives and their production waste products as sources of bioactive compounds. Current Topics in Nutrition Research, 1: 85-88.

Waksmundzka-Hajnos, M., 1998. Chromatographic separations of aromatic carboxylic acids. Journal of Chromatography B, 717: 93-118.

Wang, H., Cao, G. H., Prior, R. L., 1996. Total antioxidant capacity of fruits. Journal of Agricultural and Food Chemistry, 44: 701-705.

Wang, L., Weller, C. L., 2006. Recent advances in extraction of nutraceuticals from plants. Trends in Food Science and Technology, 17: 300-312.

Wang, H. B., Nair, M. G., Strasburg, G. M., Chang, Y. C., Booren, A. M., Gray, J. I., DeWitt, D. L., 1999. Antioxidant and anti-inflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. Journal of Natural Products, 62: 294-296.

Waterman, P. G., Mole, S., 1994. Analysis of Phenolic Plant Metabolites. Blackwell Scientific Publications. Oxford.

Wettasinghe, M., Shahidi, F., 1999. Evening primrose meal: a source of natural antioxidants and scavenger of hydrogen peroxide and oxygen-derived free radicals. Journal of Agricultural and Food Chemistry, 47: 1801-1812.

Wolfe, K. L., Liu, R. H., 2003. Apple peels as a value-added food ingredient. Journal of Agricultural and Food Chemistry, 51: 1676-1683.

Wrolstad, R. E., 2005. Bioactive food components. In: handbook of food analytical chemistry: pigments, colorants, flavors, texture, and bioactive food components.

Xiao, W., Han, L., Shi, B., 2008. Microwave-assisted extraction of flavonoids from *Radix Astragali*. Seperation and Purification Technology, 62: 614-618

Yamakoshi, J., Saito, M., Kataoka, S., Tokutake, S., 2002. Procyanidin-rich extract from grape seeds prevents cataract formation in hereditary cataractous (ICR)/f) rats. Journal of Agricultural and Food Chemistry, 50: 4983-4988.

Zhou, H.Y., Liu, C.Z., 2006. Microwave-assisted extraction of solanesol from tabocco leaves. Journal of Chromatography A, 1129: 135-139.

Zuloaga, O., Etxebarria, N., Fernandez, L. A., Madariaga, J. M., 1999. Optimization and comparison of microwave assisted extraction and Soxhlet extraction for the determination of polychlorinatedbiphenyls in soil samples using an experimental design approach. Talanta, 50: 345-357.

APPENDIX A

CALIBRATION CURVES

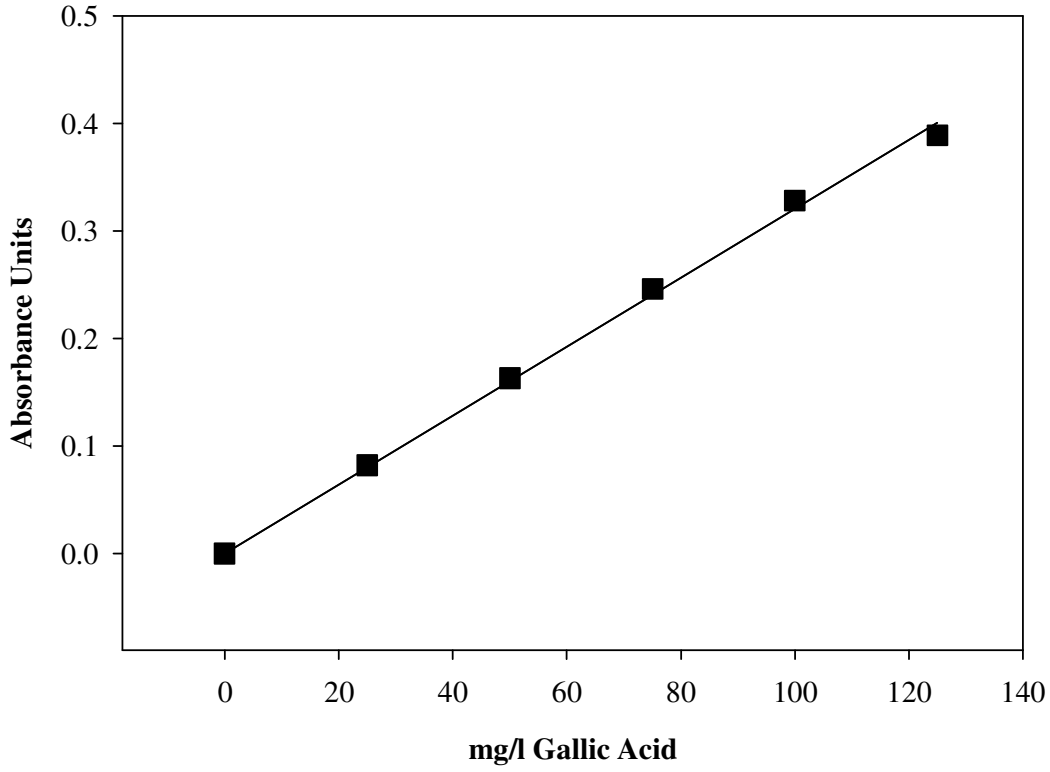


Figure A.1. Calibration curve prepared by using gallic acid and water-ethanol (1:1 v/v)

The equations for Figure A1 was expressed as

$y=0.0032x+0.0038$ $R^2= 0.998$

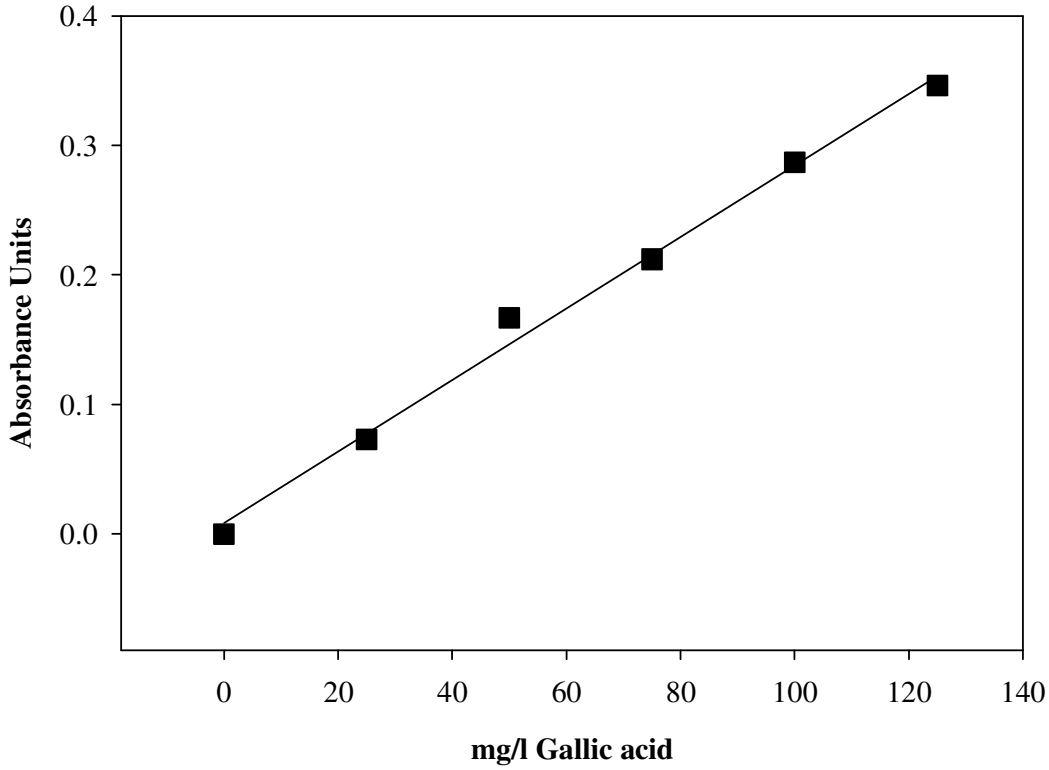


Figure A.2. Calibration curve prepared by using gallic acid and ethanol

The equations for Figure A2 was expressed as

$y=0.0028x+0.0082$ $R^2=0.993$

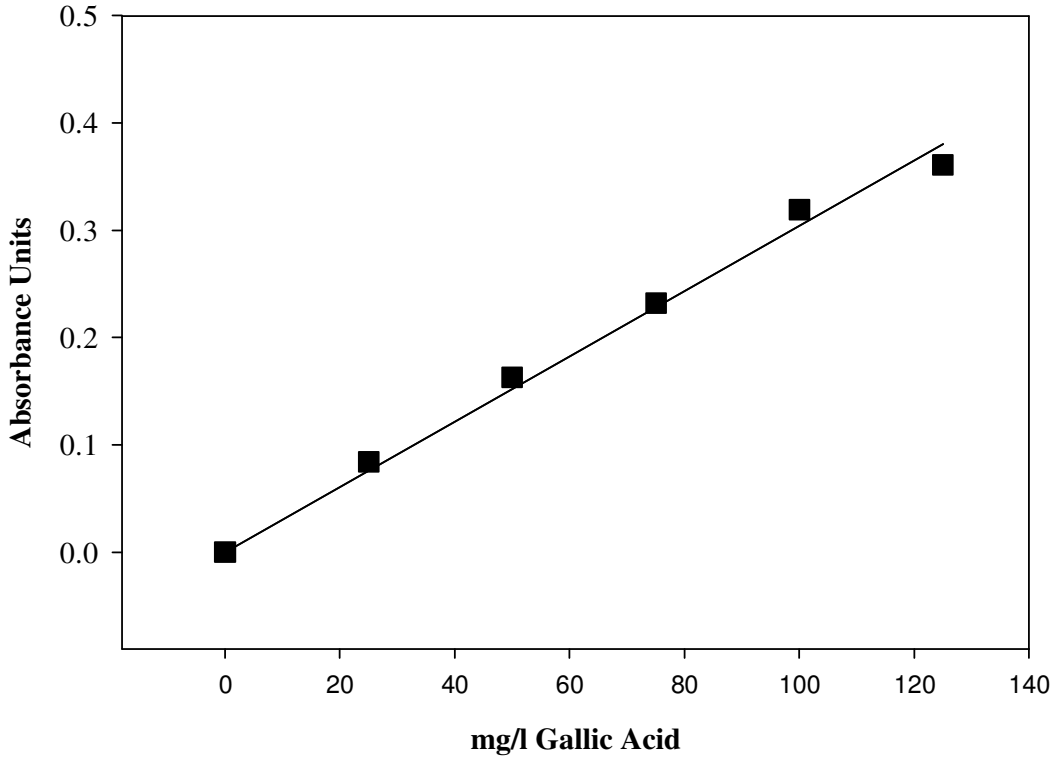


Figure A.3. Calibration curve prepared by using gallic acid and water.

The equations for Figure A3 was expressed as

$y=0.0029x+0.009$ $R^2=0.993$

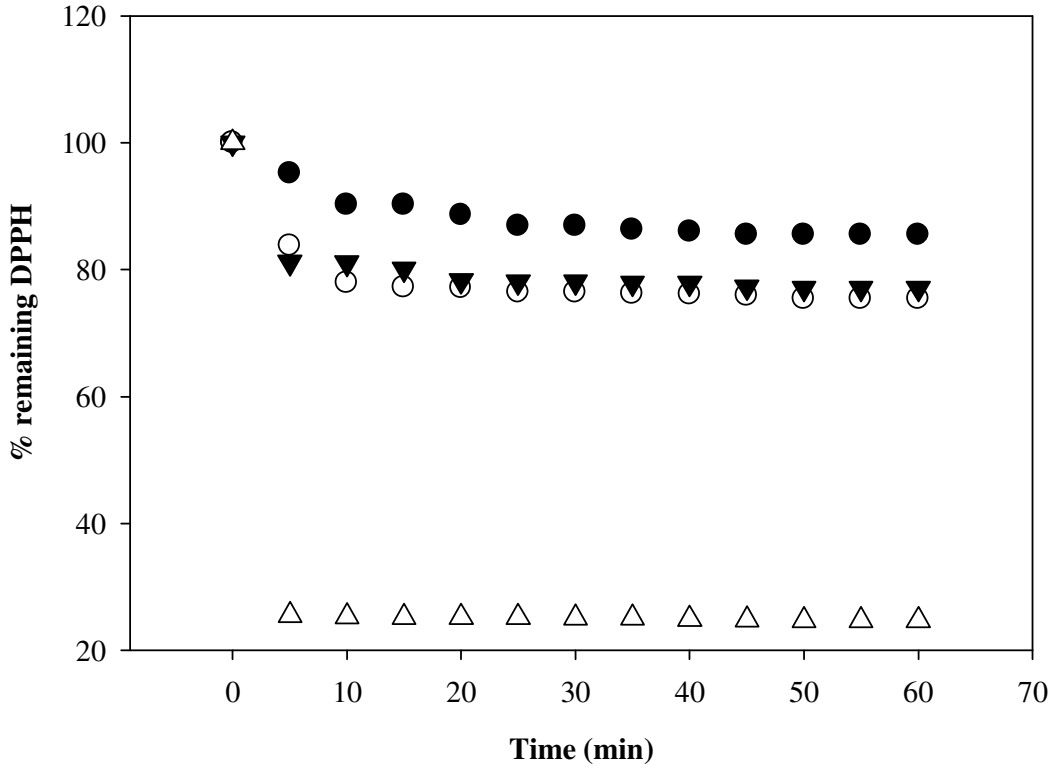


Figure A.4. DPPH˙ reduction by gallic acid as a function of time; ● 0.01 g ga/ g DPPH˙, ▼ 0.02 g ga/g DPPH˙, ○ 0.07 g ga/g DPPH˙, Δ 0.1 g ga/g DPPH˙

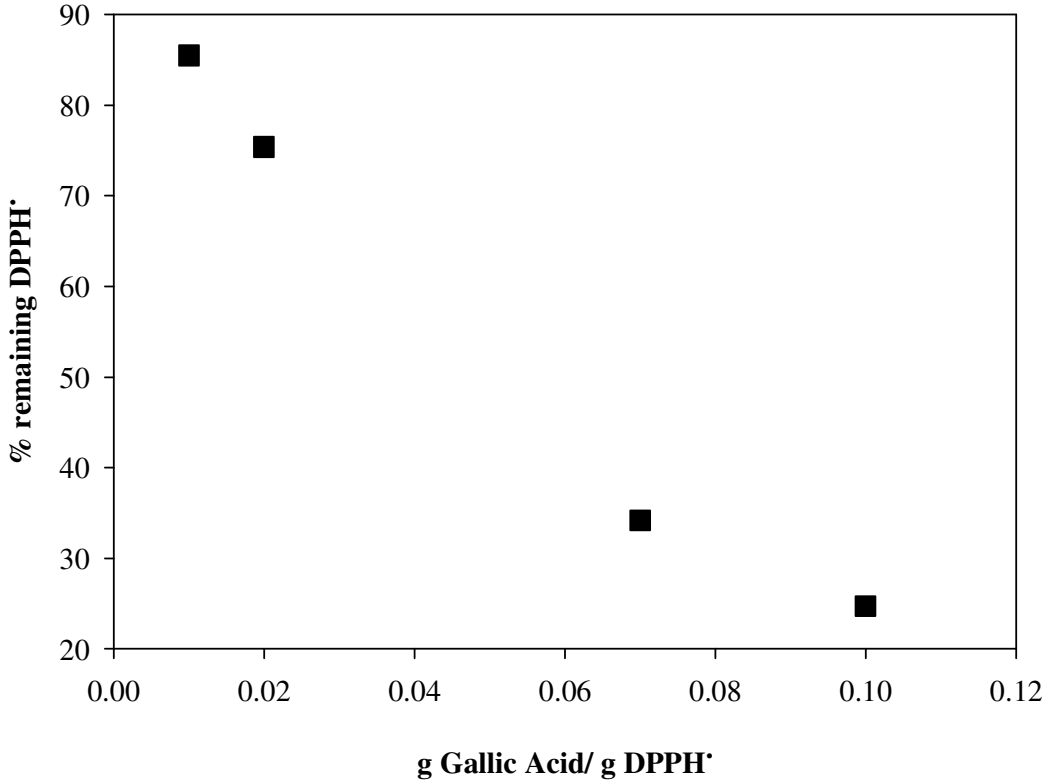


Figure A.5**.** The disappearance of DPPH· as a function of gallic acid concentration.

The equations for Figure A5 was expressed as

$y = 98.221\ e^{-14.19x}$ $R^2 = 0.995$

APPENDIX B

EXPERIMENTAL DATA

Table B.1. Experimental data of TPC and AE values of tomato pomace extracted by MAE.

Run	Solvent type	Solvent to solid ratio (ml/g)	Power (W)	Time (min)	TPC (mg GAE/g sample)	AE (mg DPPH/g sample)
1	Ethanol-water	10	400	12	1.35	1.76
2	Ethanol-water	10	400	14	1.63	2.89
3	Ethanol-water	10	400	16	1.94	4.17
4	Ethanol-water	10	400	18	1.71	3.57
5	Ethanol-water	10	700	12	1.66	3.46
6	Ethanol-water	10	700	14	2.16	4.32
7	Ethanol-water	10	700	16	1.72	3.72

Table B.1. Continued

8	Ethanol-water	10	700	18	1.72	3.72
9	Ethanol-water	20	400	12	2.70	2.94
10	Ethanol-water	20	400	14	3.08	3.25
11	Ethanol-water	20	400	16	3.61	4.52
12	Ethanol-water	20	400	18	2.76	3.48
13	Ethanol-water	20	700	12	3.32	3.42
14	Ethanol-water	20	700	14	3.76	4.84
15	Ethanol-water	20	700	16	3.26	3.85
16	Ethanol-water	20	700	18	3.26	3.85
17	Ethanol-water	30	400	12	2.64	2.86
18	Ethanol-water	30	400	14	2.88	3.02

Table B.1. Continued

19	Ethanol-water	30	400	16	3.49	4.18
20	Ethanol-water	30	400	18	3.02	3.23
21	Ethanol-water	30	700	12	2.83	3.32
22	Ethanol-water	30	700	14	3.68	4.42
23	Ethanol-water	30	700	16	2.92	3.27
24	Ethanol-water	30	700	18	2.92	3.24
25	Ethanol	10	400	12	1.28	1.68
26	Ethanol	10	400	14	1.53	2.25
27	Ethanol	10	400	16	1.92	3.45
28	Ethanol	10	400	18	1.71	2.24
29	Ethanol	10	700	12	1.31	2.74
30	Ethanol	10	700	14	1.67	3.72
31	Ethanol	10	700	16	1.56	2.77
32	Ethanol	10	700	18	1.56	2.67

Table B.1. Continued

33	Ethanol	20	400	12	2.06	2.79
34	Ethanol	20	400	14	2.13	2.86
35	Ethanol	20	400	16	3.16	3.94
36	Ethanol	20	400	18	2.91	3.42
37	Ethanol	20	700	12	2.91	2.97
38	Ethanol	20	700	14	3.49	3.98
39	Ethanol	20	700	16	2.63	3.51
40	Ethanol	20	700	18	2.63	3.51
41	Ethanol	30	400	12	2.01	2.5
42	Ethanol	30	400	14	2.12	2.62
43	Ethanol	30	400	16	2.76	3.58
44	Ethanol	30	400	18	2.44	3.12
45	Ethanol	30	700	12	2.07	2.84
46	Ethanol	30	700	14	3.03	3.72
47	Ethanol	30	700	16	2.23	3.34
48	Ethanol	30	700	18	2.18	3.34
49	Water	10	400	12	1.07	1.66
50	Water	10	400	14	1.07	1.66

Table B.1. Continued

51	Water	10	400	16	1.17	1.81
52	Water	10	400	18	1.45	2.84
53	Water	10	700	12	0.97	1.71
54	Water	10	700	14	1.17	1.94
55	Water	10	700	16	1.62	2.98
56	Water	10	700	18	1.03	2.34
57	Water	20	400	12	2.14	2.29
58	Water	20	400	14	2.28	2.41
59	Water	20	400	16	2.48	2.55
60	Water	20	400	18	3.31	3.42
61	Water	20	700	12	2.55	2.79
62	Water	20	700	14	3.10	2.82
63	Water	20	700	16	3.38	3.54
64	Water	20	700	18	3.10	2.74
65	Water	30	400	12	2.17	1.98
66	Water	30	400	14	2.17	2.31
67	Water	30	400	16	2.43	2.44
68	Water	30	400	18	2.69	3.02
69	Water	30	700	12	2.17	2.45

Table B.1. Continued

70	Water	30	700	14	2.28	2.72
71	Water	30	700	16	2.90	3.12
72	Water	30	700	18	2.69	2.47

Table B.2. Experimental data of TPC and AE values of tomato pomace extracted by conventional extraction.

Run	**Solvent type**	**Solvent to solid ratio (ml/g)**	**TPC (mg GAE/g sample)**	**AE (mg DPPH/g sample)**
1	Ethanol-water	10	1.76	3.92
2	Ethanol-water	20	3.19	4.08
3	Ethanol-water	30	3.54	4.25
4	Ethanol	10	1.25	2.78
5	Ethanol	20	2.36	3.24
6	Ethanol	30	2.88	3.68
7	Water	10	1.35	2.76
8	Water	20	2.58	2.98
9	Water	30	3.10	3.25

Table B.3. Experimental data of TPC and AE values of sour cherry pomace extracted by MAE.

Run	Solvent type	Solvent to solid ratio (ml/g)	Power (W)	Time (min)	TPC (mg GAE/g sample)	AE (mg DPPH/g sample)
1	Ethanol-water	10	400	10	10.48	21.06
2	Ethanol-water	10	400	12	10.69	22.38
3	Ethanol-water	10	400	14	11.82	23.85
4	Ethanol-water	10	400	16	9.52	22.47
5	Ethanol-water	10	700	10	10.63	22.04
6	Ethanol-water	10	700	12	11.79	24.76
7	Ethanol-water	10	700	14	11.48	22.86
8	Ethanol-water	10	700	16	11.48	22.86

Table B.3. Continued

9	Ethanol-water	20	400	10	12.70	23.83
10	Ethanol-water	20	400	12	13.17	24.52
11	Ethanol-water	20	400	14	14.17	26.84
12	Ethanol-water	20	400	16	11.89	25.58
13	Ethanol-water	20	700	10	13.20	25.72
14	Ethanol-water	20	700	12	14.14	28.32
15	Ethanol-water	20	700	14	11.42	27.67
16	Ethanol-water	20	700	16	11.42	27.67
17	Ethanol-water	30	400	10	11.41	23.73
18	Ethanol-water	30	400	12	12.72	24.25
19	Ethanol-water	30	400	14	13.85	26.49

Table B.3. Continued

20	Ethanol-water	30	400	16	12.72	25.68
21	Ethanol-water	30	700	10	10.89	24.45
22	Ethanol-water	30	700	12	13.80	27.98
23	Ethanol-water	30	700	14	12.68	26.65
24	Ethanol-water	30	700	16	12.68	26.65
25	Ethanol	10	400	10	5.58	18.84
26	Ethanol	10	400	12	5.99	19.47
27	Ethanol	10	400	14	7.72	21.32
28	Ethanol	10	400	16	6.74	20.92
29	Ethanol	10	700	10	10.56	19.97
30	Ethanol	10	700	12	11.24	22.05
31	Ethanol	10	700	14	8.58	22.01
32	Ethanol	10	700	16	8.58	22.01
33	Ethanol	20	400	10	5.99	22.66
34	Ethanol	20	400	12	6.56	23.61

Table B.3. Continued

35	Ethanol	20	400	14	9.24	25.82
36	Ethanol	20	400	16	7.45	24.92
37	Ethanol	20	700	10	9.02	24.14
38	Ethanol	20	700	12	11.27	26.18
39	Ethanol	20	700	14	9.34	25.31
40	Ethanol	20	700	16	9.34	25.31
41	Ethanol	30	400	10	4.80	21.18
42	Ethanol	30	400	12	6.14	22.76
43	Ethanol	30	400	14	9.09	24.16
44	Ethanol	30	400	16	6.89	23.78
45	Ethanol	30	700	10	8.01	23.63
46	Ethanol	30	700	12	9.46	25.77
47	Ethanol	30	700	14	8.82	24.42
48	Ethanol	30	700	16	8.82	24.42
49	Water	10	400	10	6.16	18.24
50	Water	10	400	12	6.43	19.06
51	Water	10	400	14	7.17	20.78
52	Water	10	400	16	6.91	19.16
53	Water	10	700	10	6.66	19.42

Table B.3. Continued

54	Water	10	700	12	8.43	21.08
55	Water	10	700	14	7.55	20.94
56	Water	10	700	16	7.55	20.94
57	Water	20	400	10	6.38	22.46
58	Water	20	400	12	6.90	23.12
59	Water	20	400	14	8.31	24.38
60	Water	20	400	16	8.41	23.42
61	Water	20	700	10	6.28	22.19
62	Water	20	700	12	8.93	24.46
63	Water	20	700	14	7.24	23.96
64	Water	20	700	16	7.24	23.96
65	Water	30	400	10	5.12	21.46
66	Water	30	400	12	5.95	22.02
67	Water	30	400	14	7.19	23.54
68	Water	30	400	16	6.62	23.16
69	Water	30	700	10	5.90	22.56
70	Water	30	700	12	7.97	24.43
71	Water	30	700	14	7.03	24.14
72	Water	30	700	16	7.03	24.14

Table B.4. Experimental data of TPC and AE values of sour cherry pomace extracted by conventional extraction.

Run	**Solvent type**	**Solvent to solid ratio (ml/g)**	**TPC (mg GAE/g sample)**	**AE (mg DPPH/g sample)**
1	Ethanol-water	10	11.04	18.42
2	Ethanol-water	20	12.25	23.15
3	Ethanol-water	30	13.78	24.74
4	Ethanol	10	7.64	17.13
5	Ethanol	20	8.52	19.54
6	Ethanol	30	8.09	20.08
7	Water	10	8.17	14.78
8	Water	20	9.24	15.65
9	Water	30	10.85	17.84

APPENDIX C

ANOVA AND TUKEY COMPARISON TEST TABLES

Table C.1. The representation of numbering used in the statistical analysis for MAE of tomato pomace.

Numbering	**Solvent type**	**Solvent/Solid ratio (ml/g)**	**Power (W)**	**Time (min)**
1	Ethanol-Water (1:1v/v)	10	400	12
2	Ethanol	20	700	14
3	Water	30	-	16
4	-	-	-	18

Table C.2. ANOVA and Tukey Test Table for TPC values of tomato pomace extracts obtained by MAE using different solvent types, solvent to solid ratios, power levels and time.

Between-Subjects Factors

		N
Solventtype	1	24
	2	24
	3	24
Solventtosolidratio	1	24
	2	24
	3	24
Power	1	36
	2	36
Time	1	18
	2	18
	3	18
	4	18

Table C.2. Continued

Tests of Between-Subjects Effects

Dependent Variable:TPC

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	33.112[a]	8	4.139	43.861	.000
Intercept	395.561	1	395.561	4.192E3	.000
solventtype	3.873	2	1.936	20.519	.000
solventtosolidratio	26.726	2	13.363	141.605	.000
Power	.535	1	.535	5.671	.020
Time	1.979	3	.660	6.989	.000
Error	5.945	63	.094		
Total	434.618	72			
Corrected Total	39.057	71			

a. R Squared = .848 (Adjusted R Squared = .828)

Table C.2. Continued

Solvent Type

Multiple Comparisons

TPC

Tukey HSD

(I) solventtype	(J) solvent type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.4470*	.08868	.000	.2342	.6599
	3	.5271*	.08868	.000	.3142	.7400
2	1	-.4470*	.08868	.000	-.6599	-.2342
	3	.0801	.08868	.641	-.1328	.2929
3	1	-.5271*	.08868	.000	-.7400	-.3142
	2	-.0801	.08868	.641	-.2929	.1328

Based on observed means.

The error term is Mean Square(Error) = .094.

*. The mean difference is significant at the 0.05 level.

Table C.2. Continued

TPC

Tukey HSD

solventtype	N	Subset	
		1	2
3	24	2.1415	
2	24	2.2216	
1	24		2.6686
Sig.		.641	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .094.

Tukey grouping	**N**	**Solvent type**
A	24	1
B	24	2
B	24	3

Table C.2. Continued

Solvent to solid ratio

Multiple Comparisons

TPC

Tukey HSD

(I) solventto solidratio	(J) solventtoso lidratio	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-1.4173*	.08868	.000	-1.6301	-1.2044
	3	-1.1135*	.08868	.000	-1.3263	-.9006
2	1	1.4173*	.08868	.000	1.2044	1.6301
	3	.3038*	.08868	.003	.0910	.5167
3	1	1.1135*	.08868	.000	.9006	1.3263
	2	-.3038*	.08868	.003	-.5167	-.0910

Based on observed means.

The error term is Mean Square (Error) = .094.

*. The mean difference is significant at the 0.05 level.

Table C.2. Continued

TPC

Tukey HSD

Solventtosolidratio	N	Subset		
		1	2	3
1	24	1.5003		
3	24		2.6138	
2	24			2.9176
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .094.

Tukey grouping	**N**	**Solvent to solid ratio**
A	24	2
B	24	3
C	24	1

Table C.2. Continued

Time

Multiple Comparisons

TPC

Tukey HSD

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.3330*	.10240	.010	-.6032	-.0627
	3	-.4431*	.10240	.000	-.7133	-.1729
	4	-.3267*	.10240	.012	-.5969	-.0565
2	1	.3330*	.10240	.010	.0627	.6032
	3	-.1102	.10240	.705	-.3804	.1601
	4	.0063	.10240	1.000	-.2639	.2765
3	1	.4431*	.10240	.000	.1729	.7133
	2	.1102	.10240	.705	-.1601	.3804
	4	.1165	.10240	.668	-.1538	.3867
4	1	.3267*	.10240	.012	.0565	.5969
	2	-.0063	.10240	1.000	-.2765	.2639
	3	-.1165	.10240	.668	-.3867	.1538

Based on observed means.

The error term is Mean Square(Error) = .094.

*. The mean difference is significant at the 0.05 level.

Table C.2. Continued

TPC

Tukey HSD

Time	N	Subset	
		1	2
1	18	2.0682	
4	18		2.3949
2	18		2.4012
3	18		2.5113
Sig.		1.000	.668

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is MeanSquare(Error) = .094.

Tukey grouping	**N**	**Time**
A	18	3
A	18	4
A	18	2
B	18	1

Table C.3. The representation of numbering in the statistical analysis for MAE and conventional extraction.

Numbering	**Solvent type**	**Solvent to solid ratio (ml/g)**	**Method type**
1	Ethanol-Water (1:1v/v)	10	Microwave-assisted extraction
2	Ethanol	20	Conventional extraction
3	Water	30	-

Table C.4. ANOVA Table for TPC values of tomato pomace extracts obtained by using different solvent types, solvent to solid ratios and extraction method types.

Between-Subjects Factors

		N
solventtype	1	6
	2	6
	3	6
solventtosolidratio	1	6
	2	6
	3	6
Method	1	9
	2	9

Table C.4. Continued

Tests of Between-Subjects Effects

Dependent Variable:TPC

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9.740[a]	5	1.948	27.543	.000
Intercept	119.309	1	119.309	1.687E3	.000
solventtype	1.084	2	.542	7.666	.007
solventtosolidratio	8.354	2	4.177	59.060	.000
Method	.302	1	.302	4.264	.061
Error	.849	12	.071		
Total	129.897	18			
Corrected Total	10.588	17			

a. R Squared = .920 (Adjusted R Squared = .886)

Table C.5. ANOVA and Tukey Test Table for AE values of tomato pomace extracts obtained by MAE using different solvent types, solvent to solid ratios, power levels and time.

Between-Subjects Factors

		N
solventtype	1	24
	2	24
	3	24
solventtosolidratio	1	24
	2	24
	3	24
Power	1	36
	2	36
Time	1	18
	2	18
	3	18
	4	18

Table C.5. Continued

Tests of Between-Subjects Effects

Dependent Variable:AE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	25.994[a]	8	3.249	17.285	.000
Intercept	665.334	1	665.334	3.539E3	.000
solventtype	13.347	2	6.674	35.502	.000
solventtosolidratio	3.866	2	1.933	10.283	.000
Power	2.513	1	2.513	13.366	.001
Time	6.268	3	2.089	11.115	.000
Error	11.843	63	.188		
Total	703.171	72			
Corrected Total	37.837	71			

a. R Squared = .687 (Adjusted R Squared = .647)

Table C.5. Continued

Solvent type

Multiple Comparisons

AE

Tukey HSD

(I) solventtype	(J) solventtype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.4892*	.12516	.001	.1887	.7896
	3	1.0538*	.12516	.000	.7533	1.3542
2	1	-.4892*	.12516	.001	-.7896	-.1887
	3	.5646*	.12516	.000	.2642	.8650
3	1	-1.0538*	.12516	.000	-1.3542	-.7533
	2	-.5646*	.12516	.000	-.8650	-.2642

Based on observed means.

The error term is Mean Square(Error) = .188.

*. The mean difference is significant at the 0.05 level.

Table C.5. Continued

AE

Tukey HSD

solventtype	N	Subset		
		1	2	3
3	24	2.5004		
2	24		3.0650	
1	24			3.5542
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .188.

Tukey grouping	**N**	**Solvent type**
A	24	1
B	24	2
C	24	3

Table C.5. Continued

Solvent to solid ratio

Multiple Comparisons

AE

Tukey HSD

(I) solventtosolidratio	(J) solventtosolidratio	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.5675*	.12516	.000	-.8679	-.2671
	3	-.2933	.12516	.057	-.5938	.0071
2	1	.5675*	.12516	.000	.2671	.8679
	3	.2742	.12516	.081	-.0263	.5746
3	1	.2933	.12516	.057	-.0071	.5938
	2	-.2742	.12516	.081	-.5746	.0263

Based on observed means.

The error term is Mean Square(Error) = .188.

*. The mean difference is significant at the 0.05 level.

Table C.5. Continued

AE

Tukey HSD

Solventtosolidratio	N	Subset	
		1	2
1	24	2.7529	
3	24	3.0462	3.0462
2	24		3.3204
Sig.		.057	.081

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .188.

Tukey grouping	**N**	**Solvent to solid ratio**
A	24	2
AB	24	3
B	24	1

Table C.5. Continued

Time

Multiple Comparisons

AE

Tukey HSD

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.5328*	.14452	.003	-.9142	-.1514
	3	-.8100*	.14452	.000	-1.1914	-.4286
	4	-.5589*	.14452	.001	-.9403	-.1775
2	1	.5328*	.14452	.003	.1514	.9142
	3	-.2772	.14452	.231	-.6586	.1042
	4	-.0261	.14452	.998	-.4075	.3553
3	1	.8100*	.14452	.000	.4286	1.1914
	2	.2772	.14452	.231	-.1042	.6586
	4	.2511	.14452	.313	-.1303	.6325
4	1	.5589*	.14452	.001	.1775	.9403
	2	.0261	.14452	.998	-.3553	.4075
	3	-.2511	.14452	.313	-.6325	.1303

Based on observed means.

The error term is Mean Square(Error) = .188.

*. The mean difference is significant at the 0.05 level.

Table C.5. Continued

AE

Tukey HSD

Time	N	Subset	
		1	2
1	18	2.5644	
2	18		3.0972
4	18		3.1233
3	18		3.3744
Sig.		1.000	.231

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .188.

Tukey grouping	**N**	**Time**
A	18	3
A	18	4
A	18	2
B	18	1

Table C.6. ANOVA Table for AE values of tomato pomace extracts obtained by using different solvent types, solvent to solid ratios and extraction method types.

Between-Subjects Factors

		N
solventtype	1	6
	2	6
	3	6
solventtosolidratio	1	6
	2	6
	3	6
Method	1	9
	2	9

Table C.6. Continued

Tests of Between-Subjects Effects

Dependent Variable:AE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.809[a]	5	.962	21.784	.000
Intercept	227.982	1	227.982	5.164E3	.000
solventtype	4.027	2	2.013	45.608	.000
solventtosolidratio	.518	2	.259	5.863	.017
Method	.264	1	.264	5.980	.031
Error	.530	12	.044		
Total	233.321	18			
Corrected Total	5.338	17			

a. R Squared = .901 (Adjusted R Squared = .859)

Table C.7. The representation of numbering used in the statistical analysis for MAE of sour cherry pomace.

	Solvent type	**Solvent to solid ratio (ml/g)**	**Power (W)**	**Time (min)**
1	Ethanol-Water (1:1v/v)	10	400	10
2	Ethanol	20	700	12
3	Water	30	-	14
4	-	-	-	16

Table C.8. ANOVA and Tukey Test Table for TPC values of sour cherry pomace extracts obtained by MAE using different solvent types, solvent to solid ratios, power levels and time.

Between-Subjects Factors

		N
Solventtype	1	24
	2	24
	3	24
solventtosolidratio	1	24
	2	24
	3	24
Power	1	36
	2	36
Time	1	18
	2	18
	3	18
	4	18

Table C.8. Continued

Tests of Between-Subjects Effects

Dependent Variable:TPC

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	388.325[a]	8	48.541	38.738	.000
Intercept	5964.683	1	5964.683	4.760E3	.000
Solventtype	340.476	2	170.238	135.861	.000
solventtosolidratio	9.050	2	4.525	3.611	.033
Power	19.621	1	19.621	15.658	.000
Time	19.178	3	6.393	5.102	.003
Error	78.941	63	1.253		
Total	6431.949	72			
Corrected Total	467.266	71			

a. R Squared = .831 (Adjusted R Squared = .810)

Table C.8. Continued

Solvent type

Multiple Comparisons

TPC

Tukey HSD

(I) solventtype	(J) solventtype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	3.9782*	.32314	.000	3.2025	4.7538
	3	5.0567*	.32314	.000	4.2811	5.8324
2	1	-3.9782*	.32314	.000	-4.7538	-3.2025
	3	1.0786*	.32314	.004	.3029	1.8542
3	1	-5.0567*	.32314	.000	-5.8324	-4.2811
	2	-1.0786*	.32314	.004	-1.8542	-.3029

Based on observed means.

The error term is Mean Square(Error) = 1.253.

*. The mean difference is significant at the 0.05 level.

Table C.8. Continued

TPC

Tukey HSD

Solventtype	N	Subset		
		1	2	3
3	24	7.0567		
2	24		8.1353	
1	24			12.1134
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.253.

Tukey grouping	**N**	**Solvent type**
A	24	1
B	24	2
C	24	3

Table C.8. Continued

Solvent to solid ratio

Multiple Comparisons

TPC

Tukey HSD

(I) solventtosolidratio	(J) solventtosolidratio	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.8435*	.32314	.030	-1.6191	-.0678
	3	-.2427	.32314	.734	-1.0184	.5329
2	1	.8435*	.32314	.030	.0678	1.6191
	3	.6007	.32314	.159	-.1749	1.3764
3	1	.2427	.32314	.734	-.5329	1.0184
	2	-.6007	.32314	.159	-1.3764	.1749

Based on observed means.

The error term is Mean Square(Error) = 1.253.

*. The mean difference is significant at the 0.05 level.

Table C.8. Continued

TPC

Tukey HSD

Solventtosolidratio	N	Subset	
		1	2
1	24	8.7397	
3	24	8.9825	8.9825
2	24		9.5832
Sig.		.734	.159

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.253.

Tukey grouping	**N**	**Solvent to solid ratio**
A	24	2
AB	24	3
B	24	1

Table C.8. Continued

Time

Multiple Comparisons

TPC

Tukey HSD

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-1.2120*	.37313	.010	-2.1967	-.2273
	3	-1.2796*	.37313	.006	-2.2643	-.2949
	4	-.6356	.37313	.331	-1.6203	.3491
2	1	1.2120*	.37313	.010	.2273	2.1967
	3	-.0676	.37313	.998	-1.0522	.9171
	4	.5764	.37313	.417	-.4083	1.5611
3	1	1.2796*	.37313	.006	.2949	2.2643
	2	.0676	.37313	.998	-.9171	1.0522
	4	.6440	.37313	.319	-.3407	1.6287
4	1	.6356	.37313	.331	-.3491	1.6203
	2	-.5764	.37313	.417	-1.5611	.4083
	3	-.6440	.37313	.319	-1.6287	.3407

Based on observed means.

The error term is Mean Square(Error) = 1.253.

*. The mean difference is significant at the 0.05 level.

Table C.8. Continued

TPC

Tukey HSD

Time	N	Subset	
		1	2
1	18	8.3200	
4	18	8.9556	8.9556
2	18		9.5320
3	18		9.5996
Sig.		.331	.319

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.253.

Tukey grouping	**N**	**Time**
A	18	3
A	18	2
AB	18	4
B	18	1

Table C.9. ANOVA Table for TPC values of sour cherry pomace extracts obtained by using different solvent types, solvent to solid ratios and extraction method types.

Between-Subjects Factors

		N
Solventtype	1	6
	2	6
	3	6
solventtosolidratio	1	6
	2	6
	3	6
method	1	9
	2	9

Table C.9. Continued

Tests of Between-Subjects Effects

Dependent Variable:TPC

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	85.277[a]	5	17.055	17.641	.000
Intercept	1764.972	1	1764.972	1.826E3	.000
solventtype	76.578	2	38.289	39.603	.000
solventtosolidratio	8.652	2	4.326	4.475	.035
Method	.047	1	.047	.049	.829
Error	11.602	12	.967		
Total	1861.851	18			
Corrected Total	96.879	17			

a. R Squared = .883 (Adjusted R Squared = .834)

Table C.10. ANOVA and Tukey Test Table for AE values of sour cherry pomace extracts obtained by MAE using different solvent types, solvent to solid ratios, power levels and time.

Between-Subjects Factors

		N
solventtype	1	24
	2	24
	3	24
solventtosolidratio	1	24
	2	24
	3	24
power	1	36
	2	36
time	1	18
	2	18
	3	18
	4	18

Table C.10. Continued

Tests of Between-Subjects Effects

Dependent Variable:AE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	347.693[a]	8	43.462	102.561	.000
Intercept	39480.032	1	39480.032	9.317E4	.000
solventtype	92.172	2	46.086	108.755	.000
solventtosolidratio	183.376	2	91.688	216.367	.000
Power	27.073	1	27.073	63.886	.000
Time	45.072	3	15.024	35.454	.000
Error	26.697	63	.424		
Total	39854.421	72			
Corrected Total	374.390	71			

a. R Squared = .929 (Adjusted R Squared = .920)

Table C.10. Continued

Solvent type

Multiple Comparisons

AE

Tukey HSD

(I) solventtype	(J) solventtype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	1.8188*	.18792	.000	1.3677	2.2698
	3	2.7204*	.18792	.000	2.2693	3.1715
2	1	-1.8188*	.18792	.000	-2.2698	-1.3677
	3	.9017*	.18792	.000	.4506	1.3527
3	1	-2.7204*	.18792	.000	-3.1715	-2.2693
	2	-.9017*	.18792	.000	-1.3527	-.4506

Based on observed means.

The error term is Mean Square(Error) = .424.

*. The mean difference is significant at the 0.05 level.

Table C.10. Continued

AE

Tukey HSD

solventtype	N	Subset		
		1	2	3
3	24	22.2092		
2	24		23.1108	
1	24			24.9296
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .424.

Tukey grouping	**N**	**Solvent type**
A	24	1
B	24	2
C	24	3

Table C.10. Continued

Solvent to solid ratio

Multiple Comparisons

AE

Tukey HSD

(I) solventtosolidratio	(J) solventtosolidratio	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-3.6483*	.18792	.000	-4.0994	-3.1973
	3	-3.0400*	.18792	.000	-3.4911	-2.5889
2	1	3.6483*	.18792	.000	3.1973	4.0994
	3	.6083*	.18792	.005	.1573	1.0594
3	1	3.0400*	.18792	.000	2.5889	3.4911
	2	-.6083*	.18792	.005	-1.0594	-.1573

Based on observed means.

The error term is Mean Square(Error) = .424.

*. The mean difference is significant at the 0.05 level.

Table C.10. Continued

AE

Tukey HSD

Solventtosolidratio	N	Subset		
		1	2	3
1	24	21.1871		
3	24		24.2271	
2	24			24.8354
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .424.

Tukey grouping	**N**	**Solvent to solid ratio**
A	24	1
B	24	3
C	24	2

Table C.10. Continued

Time

Multiple Comparisons

AE

Tukey HSD

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-1.5911*	.21699	.000	-2.1637	-1.0185
	3	-2.0867*	.21699	.000	-2.6593	-1.5140
	4	-1.6372*	.21699	.000	-2.2098	-1.0646
2	1	1.5911*	.21699	.000	1.0185	2.1637
	3	-.4956	.21699	.113	-1.0682	.0771
	4	-.0461	.21699	.997	-.6187	.5265
3	1	2.0867*	.21699	.000	1.5140	2.6593
	2	.4956	.21699	.113	-.0771	1.0682
	4	.4494	.21699	.174	-.1232	1.0221
4	1	1.6372*	.21699	.000	1.0646	2.2098
	2	.0461	.21699	.997	-.5265	.6187
	3	-.4494	.21699	.174	-1.0221	.1232

Based on observed means.

The error term is Mean Square(Error) = .424.

*. The mean difference is significant at the 0.05 level.

Table C.10. Continued

AE

Tukey HSD

time	N	Subset	
		1	2
1	18	22.0878	
2	18		23.6789
4	18		23.7250
3	18		24.1744
Sig.		1.000	.113

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .424.

Tukey grouping	**N**	**Time**
A	18	3
A	18	4
A	18	2
B	18	1

Table C.11. ANOVA Table for AE values of sour cherry pomace extracts obtained by using different solvent types, solvent to solid ratios and extraction method types.

Between-Subjects Factors

		N
solventtype	1	6
	2	6
	3	6
solventtosolidratio	1	6
	2	6
	3	6
method	1	9
	2	9

Table C.11. Continued

Tests of Between-Subjects Effects

Dependent Variable:AE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	219.822[a]	5	43.964	29.031	.000
Intercept	8385.557	1	8385.557	5.537E3	.000
solventtype	59.137	2	29.569	19.525	.000
solventtosolidratio	43.894	2	21.947	14.492	.001
method	116.790	1	116.790	77.120	.000
Error	18.173	12	1.514		
Total	8623.551	18			
Corrected Total	237.994	17			

a. R Squared = .924 (Adjusted R Squared = .892)