COMPARISON OF DIFFERENT ANTIOXIDANT ASSAYS FOR ESTIMATION OF ANTIOXIDANT POTENTIAL OF SELECTED FRUIT JUICE WASTE MATERIALS

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ABSTRACT

COMPARISON OF DIFFERENT ANTIOXIDANT ASSAYS FOR ESTIMATION OF ANTIOXIDANT POTENTIAL OF SELECTED FRUIT JUICE WASTE MATERIALS

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Fruit juice industry waste utilization includes several options that one of them extraction of bioactive compounds exhibiting antioxidant effect. In this study, ultrasound assisted extraction was applied to dried apple, sour cherry and grape pomaces by using ethanol, 50% aqueous ethanol and water solvents. For antioxidant capacity estimation of the extracts, $DPPH^{\bullet}$, $ABTS^{\bullet+}$, CUPRAC and FRAP assays were applied.

The highest total phenolic contents were obtained for sour cherry and grape pomace 50% aqueous ethanol extracts where the highest total phenolic content for apple pomace was obtained by water extraction. According to the FRAP method, the highest antioxidant capacities were obtained by 50% aqueous ethanol extraction and grape pomace extracts provided the highest antioxidant capacity. Similarly, the highest antioxidant capacity was determined for the grape pomace 50% aqueous ethanol extract according to CUPRAC assay. According to $DPPH^{\bullet}$ method, 50% aqueous ethanol pomace extracts provided the highest antioxidant capacity with lowest EC_{20} value and the apple pomace extract provided the highest antioxidant capacity. According to the $ABTS^{\bullet+}$ EC_{20} values, 50% aqueous ethanol extracts for grape and apple pomaces provided the highest antioxidant capacity.

FRAP and CUPRAC values were significantly ($p< 0.01$) correlated with total phenolic contents. Significant correlation between CUPRAC and FRAP assays was also obtained ($p<0.01$). Low correlations were obtained between $DPPH^{\bullet}$ EC_{20} and metal ion reducing capacity assays. The correlation was much better between $ABTS^{\bullet +}$ EC_{50} and FRAP assays especially for sour cherry pomace.

Keywords: fruit pomace, phenolic content, antioxidant capacity

ÖZ

SEÇİLMİŞ MEYVE SUYU ATIKLARININ ANTİOKSİDAN POTANSİYELİNİN BELİRLENMESİ İÇİN FARKLI ANTİOKSİDAN ANALİZLERİNİN KARŞILAŞTIRILMASI

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Meyve suyu endüstrisi atık maddelerin değerlendirme yöntemlerinden biri de antioksidan etkisi gösteren biyoaktif bileşenlerin atıklardan özütlenmesidir. Bu çalışmada, ultrason destekli özütleme işlemi etanol, 50% sulu etanol ve su çözücüleri ile kuru elma, vişne ve üzüm posalarına uygulanmıştır. Atık özütlerinde antioksidan kapasitesini belirlemek için $DPPH^{\bullet}$, $ABTS^{\bullet+}$, CUPRAC and FRAP analizleri kullanılmıştır.

Vişne ve üzüm posası %50 sulu etanol özütlerinin toplam fenolik içeriği diğer özütlere göre en yüksek değer olarak belirlenirken, toplam fenolik içeriği elma posası için su özütünde en yüksek olarak bulunmuştur. FRAP yöntemine göre, en yüksek antioksidan kapasitesi %50 sulu etanol özütlerinden elde edilmiş ve üzüm özütleri en yüksek antioksidan kapasitesi değerini göstermiştir. Benzer olarak, CUPRAC yöntemi kullanılarak %50 sulu etanol çözücüsü ile elde edilmiş üzüm posası özütünün, tüm örnekler içerisinde en yüksek antioksidan kapasitesine sahip olduğu belirlenmiştir. $DPPH^{\bullet}$ yöntemine göre, %50 sulu etanol özütleri en düşük EC_{20} değeri ile en yüksek antioksidan kapasitesi değerini sağlamıştır. Fakat en yüksek antioksidan kapasitesi elma posası özütünden elde edildi. $ABTS^{\bullet+}$ EC_{20}

değerlerine göre, elma ve üzüm 50% sulu etanol özütleri en yüksek antioksidan kapasitesi değerini sağladı.

FRAP ve CUPRAC değerleri, toplam fenolik içeriği ile önemli ölçüde ($p< 0.01$) korele olarak bulunmuştur. CUPRAC ve FRAP testleri arasında da önemli korelasyon elde edilmiştir ($p<0.01$). Buna karşın $DPPH^{\bullet}$ EC_{20} ile metal iyonu azaltma kapasitesi deneyleri arasında düşük korelasyonlar elde edildi. $ABTS^{\bullet+}$ $EC5_{0}$ ve FRAP testleri arasında özellikle vişne posası için korelasyon çok daha iyi olarak belirlenmiştir.

Anahtar Kelimeler: meyve posası, fenolik miktarı, antioksidan kapasitesi

To my family

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LIST OF ABBREVIATIONS

ABBREVIATIONS	
GA	Gallic acid
GAE	Gallic acid equivalent
FR	Free radical
ROS	Reactive oxygen species
TEAC	Trolox equivalent antioxidant capacity
FRAP	Ferric ion reducing antioxidant power
CUPRAC	Cupric ion reducing antioxidant capacity
ABTS	2,2'- Azino – Bis – 3 –Ethylbenzothiazoline -6 Sulfonic cid
DPPH	2,2 – Difenil – 1 Pikrildirazil
UAE	Ultrasoud-assisted exractiom
HAT	Hydrogen atom transfer
TPC	Total phenolic content
SC	Scavenging capacity
SET	Single electron transfer

CHAPTER 1

INTRODUCTION

Fruits and vegetables are important source of nutrients for human health and diet in daily life. Therefore, their production is increasing due to the rising population and changing dietary habits in the world (Schieber et al., 2001; Vilarino et al., 2017). Higher production than required, loss due to insufficient handling techniques and their processing wastes are important economical and environmental problems (Sagar et al., 2018). According to the Food and Agriculture Organization of United Nations (FAO), 1.3 billion tons of food produced in world is lost and wasted per year (FAO, 2014). Waste are unconsumed part of the fruit and vegetables such as leaves, seeds, peels and pomace. %25 to %30 of the fruits and vegetables are remained as a waste material after which are not further used (Ajila et al., 2007, 2010). Fruit and vegetable juice industry produce 5.5 million tons of pomace waste. In addition, grape and wine industries also produce 5 to 9 million tons of pomace for every year in the world (Schieber et al, 2001).

Utilization of plant based wastes is important because of the presence of bioactive compounds such as phenolic compounds. Phenolic compounds have health benefits for human body due to their antioxidant properties (Rudra et al, 2015). Therefore, the characterization and recovery of phenolic compounds and determination of their antioxidant properties have an important role for reducing food loss and waste materials.

1.1 Phenolic Compounds

Phenolic compounds are plant secondary metabolites and responsible for sensory characteristics and the nutritional quality improvement of fruits and vegetables (Tomas -Barberan et al., 2000; Lapornik et al., 2005). They are the largest classes of the bioactive compounds and have important biological functions (Sagar et al., 2018). They have at least one hydroxyl group attached directly to the aromatic ring. Hydrogen atom is labile in hydroxyl groups that makes phenols weak acids. Phenolic compounds are generally found as a esters or glycosides (Vermerris & Nicholson, 2006).

1.1.1 Classification of Phenolic Compounds

Phenolic compounds are the largest group among bioactive compounds. There are different ways to categorize phenolic compounds. Simple phenolics have one or more hydroxyl group attached to an aromatic ring. Phenol is the simplest compound in this group. The compounds that have one or more hydroxyl group attached to one or more aromatic rings are described as poly-phenols (Vermerris & Nicholson, 2006). Lignans, phenolic acids, flavonoids and stilbenes are the subgroups of polyphenols (Figure 1.1)

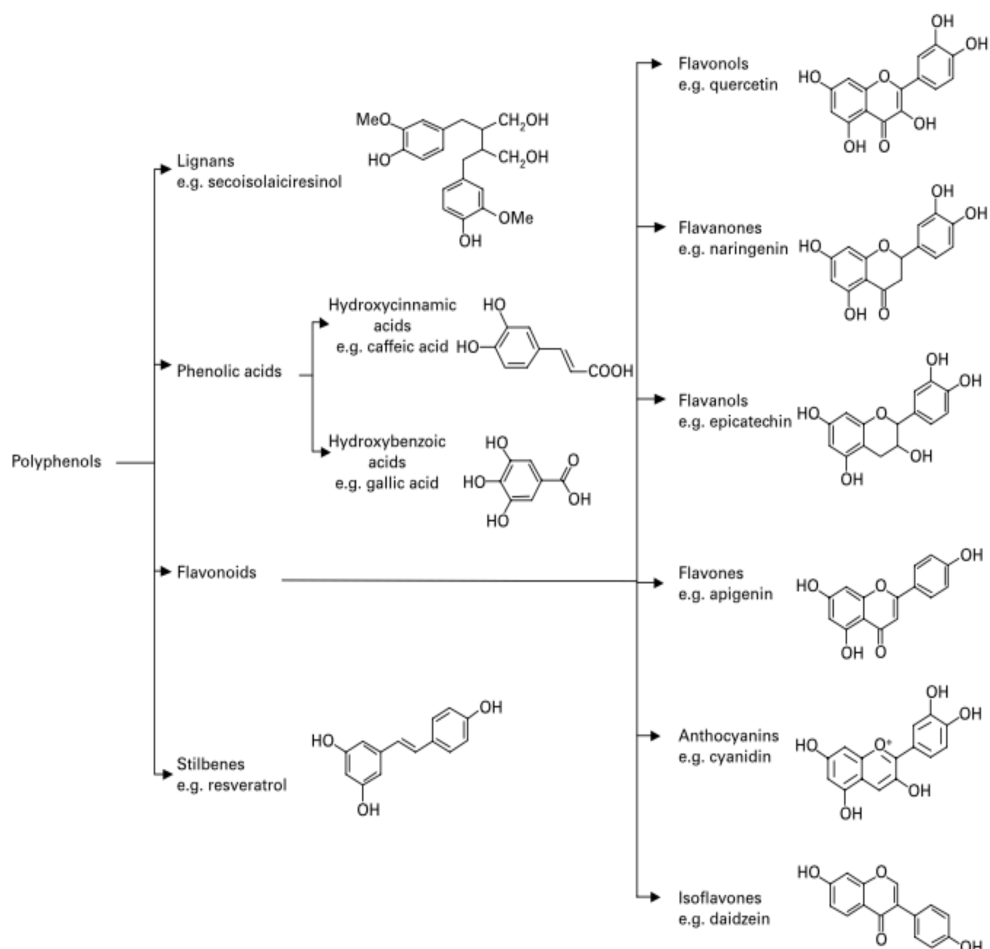


Figure 1.1 Classification and chemical structure of major classes of polyphenols (Spencer et al., 2008).

1.1.1.1 Phenolic Acids

Phenolic acids have an aromatic ring structure with one or more hydroxyl substituent (Mattila et al., 2006). Hydroxybenzoic acid and hydroxycinnamic acid are the main subgroups of phenolic acids. Hydroxycinnamic acids include p-coumaric, caffeic, ferulic and sinapic acids. Phenolic acids are generally present in the bound form. Hydroxycinnamic acids appear as simple esters with quinic acids, skihimic and tartaric acid or glycosylated derivatives. Many fruits include caffeic acid which is the most abundant phenolic acids (%75 and %100 of the total

hydroxycinnamic acid content). High amount of ferulic acid is found in the outer part of the cereal grains. Chlorogenic acid is the combined from quinic and caffeic acids and found in several fruits. (Manach et al., 2004)

Hydroxybenzoic acids have carboxyl group substituted on a phenol. Hydroxybenzoic acids include gallic acid, p-hydroxybenzoic acid, protocathechuic acid, salicylic acid and vanillic acid (Vermerris & Nicholson, 2006). Hydroxybenzoic acid content of edible plant is very low except onion, black radish and certain red fruits. In addition, hydroxybenzoic acids are components of hydrolysable tannins that have complex structures (Manach et al., 2004). Phenolic acids are not abundant in most plants except gallic acid (Vermerris & Nicholson, 2006). Tea is the main source of gallic acid that is well known phenolic acid in this group. Also, gallic acid can found in some wood plants such as oak and chestnut (Verma et al., 2013). Gallic acid is precursor of gallotannins and ellagitannnins (Vermerris & Nicholson, 2006)

1.1.1.2 Flavonoids

This phenolic group is C_{15} compounds that their structures are C_6-C_3-C_6 (Vermerris & Nicholson, 2006). A group of 3 carbons links two benzene ring. Flavonoids include 6 subclasses (Figure 1.1). They are flavonols, flavanols, flavones, flavanones, anthocyanins and isoflavones.

Flavonols are most abundant class of flavonoids in foods. Quercetin and kaempferol are well known compounds in this group. They are generally exist in foods in low concentrations. Light is effective on their biosynthesis. Therefore, they are intensively present on the outer surface, skin and leaves of the fruit. Even there are differences between concentration of flavonol in the different sides of fruit parts according to sunlight exposure. Flavonols which are present in tea and red wine are glycosylated form. Different flavonol glycosides are often available in fruits (Price et al, 1995).

Flavones including double bond at 2- 3 positions, have similar structure with that of flavonols. However, they have no hydroxyl group at 3 position (Karakaya, 2004). They are less common than flavonols. They generally include glycoside of luteolin and apigenin (Manach et al., 2004). Furthermore, the skin of the citrus fruits is rich in poly-methoxylated flavones which are most hydrophobic such as tangeretin, nobiletin and sinensetin (Shahidi & Naczk, 1995).

Flavanones are present in tomatoes and some aromatic plant like mint and also their concentrations are low. Only citrus fruit have high concentration of flavanones. Naringenin, hesperidin and eriodictyol are the main agylcones which are found in grape fruit, oranges and lemons (Manach et al., 2004). They impart the flavor of the citrus fruits. For instance, naringenin gives a bitter taste to grape fruit (Peterson & Dwyer, 1998).

Isoflavones have structural similarities to estrogens. They are classified as phytoestrogens because of that they have ability to bind to estrogen receptors. Their main sources are leguminous plant like soya and its products. They have 3 main members: genistein, daidzein and glycitein. They are heat sensitive molecules and hydrolyzed to glycosides during processing (Manach et al., 2004).

Flavanols are one of the class in flavonoids. They are not present in glycoside form like other flavonoids (Van der Sluis, 2005). They can be found as a monomer (catechin) and polymer form (pro-anthocyanidins). Green tea and chocolate are the main sources of catechins. Catechins are presents in red wine and many types of fruits. The flavins (dimer) and the arubigins (polymer) are more condensed polyphenols which are formed as a result of oxidation of flavanols monomer that are present in black tea during heating process of tea leaves. On the other hand, black tea seeds of leguminous plants and especially tea contain gallocatechin, epigallocatechin and epigallocathin gallate (Manach et al., 2004). Pro-anthocyanidins which are called condensed tannins are especially found in some fruits and their juices and naturally present in cereal, legumes seeds. They are dimers, oligomers and polymers of catechin (Manach et al., 2004). Because of the

polymeric structure of pro-anthocyanidins, their estimation and analysis are difficult. They are responsible for astringency flavor of several types of beverages, fruits and also bitterness for chocolate. They have good reducing capacity. Furthermore, they can form stables complexes with proteins and metal ions (Santos-Buelga & Scalbert, 2000).

Anthocyanins are very large group in flavonoids which are found in glycosides form (Karakaya, 2004). They are water soluble pigment dissolved in plant's the vacuolar sap of the epiderman tissues (Mazza & Miniati, 1993). They are hydrophilic secondary metabolites which impart a blue, red, orange color for fruits such as apples and grapes (Jezek et al., 2018). Red wine, cereals and leafy vegetables are rich in anthocyanins. According to the pH, they can be found in colored and uncolored form. Anthocyanins are resistant to light, pH and oxidation when they are found in plant but they are generally unstable in aglycones form (Manach et al., 2004). Cyanidin is the well-known anthocyanin in this group.

1.1.1.3 Plant Source of Polyphenols

Fruit and vegetables are the main sources of polyphenols. It is difficult to estimate the exact phenolic composition of fruits and vegetables, since a large number of different compounds presents.

1.1.2 Ultrasound Assisted-Extraction of Phenolic Compounds

Determination of the polyphenol content and also the recovery of phenolic compounds from fruit and vegetable products can be influenced by the extraction method applied. For the improvement of conventional extraction methods (e.g. percolation, decoction, heat reflux extraction, Soxhlet extraction and maceration), more rapid, efficient and effective extraction techniques have been applied for the extraction of polyphenols from plant matrices. Supercritical fluid extraction, ultrasound-assisted extraction (UAE), microwave-assisted extraction and

pressurized liquid extraction methods are examples that are used instead of classical conventional extraction methods (Ameer et al., 2017). Low frequency/high power UAE is a rapid, reproducible, clean and innovative extraction method. During the application of ultrasound in the kilohertz range, local hotspots at macroscopic scale are generated with high shear stress and temperature by producing cavitational bubbles. When the cavitation bubbles burst at the surface of the plant sample matrix, they cause an induced damage to plant cell wall and enhance the mass transfer of phenolic compounds across cellular membranes into the solvent. Sonication probes or an ultrasonic bath can be used to perform extraction. Sonication probes are commonly preferred compared to bath system due to the higher ultrasonic intensity (Kumar et al., 2021). Extraction time, solvent type, and input power are other important factors affecting UAE extraction efficiency. Although the ultrasonic waves have been reported to result in the degradation of some phenolic acids and the creation of highly reactive hydroxyl radicals within the gas bubbles, UAE was generally reported as an effective extraction technique (Galanakis, 2015; Wani et al., 2021; Khadhraoui et al., 2021; Sridhar et al., 2021)

Researchers worked on polyphenol recovery from food materials or food processing wastes by comparing different extraction methods including UAE. For instance, 50 % increasing polyphenol extraction yield from apple pomace was obtained by using combination of ultrasonics at 35 kHz and conventional extraction at 70 °C (Corrales et al., 2008). In another study, Soxhlet extraction and UAE were used for extraction polyphenols from grape seeds. The results showed that the extraction yield of polyphenols were same for both extraction techniques, but the extraction time of Soxhlet method (6 hours) is much more longer than that of UAE (0.5 hours) (Da Porto et al. 2013). Eh &Teoh (2012) used UAE to increase the extraction of lycopene, lipophilic antioxidants, from tomato waste. The extraction yield was increased by 26% compared with that of conventional extraction. Furthermore, the higher isoflavones extraction yield was obtained by using UAE (650 W) than that of conventional solvent extraction at the same conditions by

using 50% ethanol as solvent. Similarly, UAE was used for extraction of phenolic acids from Satsuma mandarin peels and higher extraction yield of phenolic acids were obtained by using UAE than those obtained by convectional maceration extraction (Xu et al., 2007). In the study of Ghafoor et al. (2009), the UAE of anthocyanins and total phenolics from grape seed was optimized in terms of extraction conditions. According to these researchers, the process time was determined the most significant parameter for the improvement the extraction yield of anthocyanins. The optimum condition of anthocyanin extraction was found to be as 29 min with 53% ethanol solvent. Wiktor et al., (2016) found that the TPC and antioxidant capacity for apple tissue were 145.3 and 64.5% higher than that of the control (without ultrasound treatment), respectively. They applied ultrasound to the sample through a water medium in the bath as well as in the beaker and direct irradiation showed a stronger effect on phenolic extraction. Based on the results in the study of Pollini et al. (2021), UAE was the best extraction technique to obtain phenolics from fresh Red Delicious apple pomace by using 50 % ethanol with a solid to solvent ratio of 1:10 g/mL at 60°C for 60 min. In the study by Goula et al. (2016), the best grape pomace polyphenol extraction yield (9.57 mg of gallic acid/g of dry mass) was obtained using UAE for 10 min whereas only 0.32 mg of gallic acid/g of dry mass was extracted in optimal conditions using indirect UAE for 25 min. UAE was also provided a higher yield of phenolic compounds as compared with that of traditional extraction techniques.

During extraction, ultrasound effect is known as decreased at temperatures higher than 50°C. The mechanical energy is converted into heat during the propagation of sound in the solvent, resulting in solvent heating. Inadequate temperature control may result in significant deviations of extraction results, especially in the case of high power ultrasound application. Therefore, an external temperature control system is required. UAE can be successfully used for the extraction of polyphenols from whole plant matrices and their by-products. UAE provides the extraction of polyphenols in short time, at low temperature, with lesser energy and sol vent

requirement. For this extraction technique, frequency, power, cycle, temperature, time, solvent type, solid- liquid ratio were important parameters.

1.2 Phenolic Content of Sour Cherry, Grape and Apple

Phenolic compounds are widely found in fruits that are important in human diet due to beneficial health effects. Contribution of fruit-processing industry is more than 0.5 billion tons of global waste worldwide. Fruit processing wastes such as peel, pomace and seeds, are rich in bioactive compounds. Therefore, the use of these waste materials as a source of polyphenols may provide economic benefits to food processors (Siracusa & Ruberto, 2019). Thus, researchers have been carried on different studies related to the fruit source, extraction, characterization and use of phenolic compounds in different product formulations.

1.2.1 Phenolic Content of Apple and Apple Pomace

Main phenolic compounds found in apples and apple pomace are hydroxycinnamic acids, dihydrochalcone derivatives (specially phloridzin), flavan-3-ols (catechin as monomers or procyanidins as oligomers), flavonols (rutin, quercetin and quercetinglycosides) and anthocyanins. There are different distributions of phenolics in different parts of the fruit, such as the flesh, seeds, leaves, and skin. The skin is rich in flavonols and anthocyanins (Lommen et al., 2000; Schieber et al.,2001; Perussello et al., 2017; Kruczek et al., 2017). Phenolic acids in apple pomace are mainly chlorogenic, caffeic, ferulic, p-coumaric, sinapic and p-coumaroyl-quinic acids in the range of 523–1542 mg/kg dry pomace. Isorhamnetin, kaempferol, guercetin, rhamnetin, glycoconjugates, procyanidinB2 and epicatechin are main flavonoids reported in the range of 2153–3734 mg/kg dry pomace. Lyu et al. (2020) reported that cyanidin-3-O-galactoside was the major anthocyanin in the range of 50–130 mg/kg dry pomace.

According to different studies of polyphenols in apple and apple pomace, phenolic content differs among apple cultivars. Phenolics in fruits are in both soluble and bound forms that is in the form of β-glycosides. Vinson et al. (2001) reported that 51.9 % of total phenols were the bound ones for apples (unknown cultivar) with lower contribution to phenolic content estimation. But, Sun et al. (2002) reported that phenolics in fruits were mainly in soluble free form that is 91.8 % for apples (unknown cultivar).

Tsao et al. (2003) studied with eight white-fleshed apple varieties. Total phenolics were in the range of 1016.5 to 2350.4 mg GAE /kg fresh weight in the peel that was higher than the phenolic content of flesh (177.4 to 933.6 mg/kg fresh weight). Wojdyło et al. (2008) studied the phenolic content of 67 different apple varieties and their phenolic content. Procyanidins were the most abundant phenolic group in apple (%80 of total phenolics) and the procyanidin content was ranged from 4622.1 to 2548.0 (mg/kg dry weight) and Golden Delicious variety had lowest procyanidin content. Hydroxycinnamic acid content of 22 varieties have found in a range of 1000-3500 mg /kg dry weight. Moreover, total flavonols in apple varieties were found in range of 80 -1660 mg/kg dry weight in the same study. Changes in TPC during apple growth were investigated from the 7th to 140th day after full bloom by Mureşan et al., (2012). Phenolic content varied between 57.76 -1224.40 mg GAE /100g for Golden Delicious apples. Diñeiro García et al. (2009) worked on eleven different cider apple pomaces, including six from single-cultivar and five from cider making industry. Major phenols in apple pomaces were reported as flavanols, dihydrochalcones flavonols and cinnamic acids (chlorogenic and caffeic acids). The pomace TPCs were ranged between 2.3 - 15.1 g GAE /kg dry matter.

Reported phenolic contents were strongly affected by the extraction method applied. For example, Wiktor et al. (2016) found that the TPC and antioxidant capacity for apple tissue were 145.3% and 64.5% higher than that of the control (without ultrasound treatment), respectively. Reis et al. (2012), analyzed apple pomace water extracts. Water successfully extracted flavones, flavonols, flavanols,

dihydrochalcones, and hydroxycinnamic acids, but not being efficient to extract quercetin glycosides. The phenolic compounds identified by these authors were kaempferol, quercetin, isorhamnetin, quercetin 3-O-arabinoside, quercetin 3-Oglucoside, quercetin 3-O-rhamnoside, quercetin 3-O-galactoside and rutin, epicatechin, procyanidin dimer A2, procyanidin dimer B1 or B2, procyanidin trimer C, procyanidin tetramer D, phloretin, phloridzin, phloretin 2'-O-xylosyl-glucoside, hydroxycinnamic acids, chlorogenic acid, and feruloyquinic acid.

Zhang et al. (2016) reported TPC of the five extracts (methanol, ethanol, acetone, ethyl acetate and chloroform) varied significantly according to the extraction medium, ranging from 1.62 to 3.05 mg GAE/g powder (1 g of powder was extracted with 5 ml of solvent in ultrasonic bath at 37 °C for 40 min) The highest level of phenolics was detected in the methanol extract, whereas the lowest was observed in the chloroform extract. Extraction with methanol and ethanol showed insignificant differences, since the polarity and solvency of methanol and ethanol were extremely similar. Rana et al. (2021) investigated, pomace of five apple varieties: Royal Delicious, Golden Delicious, Red Chief, Red Delicious and Red Gold for proximate composition, phenolic constituents and antioxidant capacity. The total soluble content of apple pomace samples was ranged from 2.05 ± 0.07 to 5.00 ± 0.01 %. The highest total soluble content was recorded for Golden Delicious apple pomace. The TPCs were measured between 2.19 ± 0.09 and 4.59 ± 0.47 mg GAE/g dry weight that it is 2.7 mg GAE/g dry weight for Golden Delicious apple pomace. In their study, the pomace samples were dried at 60°C, ground (1 mm) and then extracted with 70% aqueous methanol (1 g powder/20 ml) at 60°C for 30 min to obtain polyphenol enriched extracts. Uyttebroek et al. (2017) studied freeze-dried Golden Delicious apple pomace for the extraction of phenolic compounds with antioxidant properties. Extraction was done with 56 % ethanol (10 g dry solid / L solvent) at 80°C 56% for 31 min. extraction time. The total concentration of the 10 marker phenolic compounds (quinic acid, cathechin, epicathechin, chlorogenic acid, avicularin, phlorizin, quercitrin, hyperin, isoquercitrin, procyanidin and rutin) was 5.098 + 0.075 mg /g DW. The phenolic content of freeze-dried Golden

Delicious apple pomace 80% methanol extract was determined 6.8 ± 1.2 mg GAE/100 g DW according to the study of Maragò et al., (2015).

Krasnova I. and Segliòa (2019) were also applied water extraction that 200 g of fresh apple pomace from 11 apple cultivars were mixed with 800 ml hot water at an initial 95°C temperature and macerated for 10 h. Phenolic compounds transferred from apple pomace to water extracts are in the range of 2.9–9.6 % of total phenols depending to apple cultivar (14.04-27.97 mg GAE/ 100 g DW).

Gonelimali et al. (2021) showed that drying conditions were important since they could affect phenolic composition of the pomace during extraction. Based on their results, drying apple pomace at 60°C resulted in significantly higher phenolic content. The other aspect to be considered is the possible effect of polyphenol oxidase presence in the pomace. The enzyme was suggested to be optimally activated at 50°C and to stop or slow down this effect, it is necessary to dry the apple pomace immediately upon the production (Kammerer et al., 2014).

1.2.2 Phenolic Content of Grape and Grape Pomace

Grape is the largest fruit crop in the world. Grape pomace, a mixture of skins, seed and stems, is approximately 20 % of the grapes and produced from wine or juice processing. Grape and its pomace are rich sources of polyphenolic compounds including anthocyanins, flavonols, flavanols, phenolic acids and stilbenes (e.g.resveratrol) (Tseng & Zhao, 2012). Arts et al. (2000) reported total catechin content of several fruits. Catechin was determined as 203.9 mg/kg fresh weight in black grape that was the highest value obtained among the fruits analyzed.

Phenolic compounds can be in either free form or bound form. The extraction of bound phenolics is difficult since they are hindered by the cell wall structure of grape. Therefore, the extraction method can greatly affect the extraction yield of phenolics (Khoddami et al., 2013). Grape seed and skin extracts from Cabernet

Sauvignon, Kalecik Karası and Narince grape cultivars were analyzed for phenolic compositions by Baydar et al. (2011). The defatted grape seed powder and powdered skin were extracted in a Soxhlet apparatus for 8 h with 200 ml of acetone: water: acetic acid (90:9.5:0.5) at 60°C. TPCs varied from 522.49 to 546.50 mg GAE/ g in dry seed extracts and from 22.73 to 43.75 mg GAE/g in dry skin extracts. Librán et al., (2013) studied solid-liquid extractions of Tempranillo red grape pomace at room temperature with 1/25 (w/v) sample to solvent ratio. Results ranged from 4.58 to 28.06 mg GAE/g dry sample, depending on the solvent type. The highest phenolic content was obtained with 75% ethanol. Anthocyanins were the most abundant phenolics presenting approximately 40% of the total polyphenols extracted.

Luchian et al., (2019) showed that extraction with 50% ethanol (2 g dry pomace/5 ml solvent; 24 h stirring at room temperature) provided better results for the determination of phenolic compounds from grape pomace of Sauvignon Blanc, Traminer, Busuioac˘a de Bohotin, Cabernet Sauvignon, Merlot, Feteasc˘a Neagr˘a and Feteasc˘a Regal˘a grape varieties. The results shows a variation in the range of 2.03 - 2.78 mg GAE/ ml extract. Rajha et al. (2014) reported that the accelerated solvent extraction (100 bars pressure; 38 g solid/100 mL extraction cell; 15 min) of phenolic compounds from wet and dried Cabernet Sauvignon grape pomace, provided the highest phenolic compounds yield for wet (162 mg GAE/g dry matter) and dry (728 mg GAE/g dry matter) grape pomace extracts with 70% ethanol solvent at 45°C.

Brezoiu et al. (2019) studied the polyphenolic extraction from two red grape pomaces from the Black Sea region (Cabernet Saugvinon, and Feteasca Neagra) by using conventional extraction (pomace/ethanol ratio of 1/6 (w/v) for three times for 1 h). The total polyphenolics content was determined after ascorbic acid correction as 24.33–53.14 mg GAE / gram of vegetal material. Gallic acid (0.462–1.171 mg/g extract), vanillic acid (0.368–1.088 mg/g extract), syringic acid (0.339–2.031 mg/g extract) and protocatechuic acid (0.104–0.489 mg/g extract) contents were

determined as high, whereas resveratrol was determined in low concentrations (0.019–0.083 mg/g extract).

Carmona-Jiménez et al., (2018) determined TPC in a concentration range of 13.73–16.38 mg of GAE / g of fresh grape pomace for five different grape pomaces (Tempranillo, Tintilla de Rota, Cabernet Sauvignon, Petit Verdot and Syrah). For extraction, sonification (200 W, 24 kHz) was applied for 6 min and 50% ethanol was used as solvent. The phenolic compounds determined were flavan-3-ols, flavonols and anthocyanins. Drying as a pretreatment, provided an increase in the extractability of these compounds. Iora et al., (2015) reported that TPCs of grape pomace varied between 30.15 and 51.02 mg GAE/ g extract for different grape varieties. The Cabernet Sauvignon pomace had the highest TPC. In this study, the extraction was carried out with Falcon tubes in a rotating mixer for 24 h with 40% of ethanol for a solute/solvent ratio of 1:20 (w/v).

Agustin-Salazar et al. (2014) compared different solvents for the extraction of phenolic compounds from grape pomace. The highest extraction yield was obtained with 50% aqueous ethanol in the order of: water < ethanol < 70% aqueous methanol < 70% aqueous ethanol <50% aqueous ethanol. Since the addition of water to organic solvents provides a more polar medium, which facilitates the extraction of polyphenols.

1.2.3 Phenolic Content of Sour Cheery and Sour Cherry Pomace

Sour cherry has a characteristic astringent taste and this limits the fresh consumption. Therefore, it is mainly used in fruit juice industry. Cherries are one of the best sources of anthocyanins. Cyanidin 3-sophoroside, cyanidin 3-glucosylrutinoside, cyanidin 3-glucoside and cyanidin 3-rutinoside are main anthocyanins in sour cherry. Moreover, sour cherry contains cyanidin 3-arabinosylrutinoside, pelargonidin 3-glucoside, and peonidin 3-rutinoside. Sour and sweet cherries have hydroxycinnamates such as neochlorogenic acid and p-

coumaroylquinic acid. Flavonols and flavan-3-ols were also determined in sweet and sour cherries. The most well- knowns are catechin, epicatechin, quercetin 3-glucoside, quercetin 3-rutinoside, and kaempferol 3-rutinoside (Chandra et al., 2001; Gonçalves et al., 2004; Chaovanalikit & Wrolstad, 2004). Yilmaz et al. (2015) reported that cyanidin-3-glucosyl-rutinoside, neochlorogenic acid, and catechin were the most abundant phenolic compounds found in the sour cherry pomace. Total phenolic, anthocyanin, cyanidin-3-glucosylrutinoside, neochlorogenic acid and catechin contents were determined as 14.23 ± 0.38 mg GAE /g dry sample, 0.41 ± 0.02 mg cyanidin-3-rutinoside equivalent/g dry sample, 0.19 ± 0.02 mg/g dry sample, 0.22 ± 0.01 mg/g dry sample and 0.22 ± 0.02 mg/g dry sample in the pomace at optimum solid-liquid extraction conditions (51% ethanol; 75°C; 12mL/g solvent to solid ratio; 100 min), respectively.

Kim et al. (2005) studied on various sweet and sour cherry cultivars and reported that the TPCs of sweet and sour cherry cultivars were 109.8 and 228.9 mg GAE/100 g fresh cherry, respectively. Total anthocyanin content in sour cherry was measured as 49.1 - 109.2 mg cyanidin 3-glucoside equivalents /100 g fresh cherry that was about 1.6 times higher than total anthocyanins in sweet cherries.

In the study of Milić et al. (2021), dry sour cherries were extracted by UAE. Temperature (40–80°C), ethanol concentration (40–80%, w/w), liquid–solid ratio (10–30 mL/g) and extraction time (20-40 min) were independent variables. TPC of the extracts were in the ranfe of 1.16 to 1.96 g GAE/100 g dry sample.

Demirdöven et al. (2015) reported the phenolic and anthocyanin contents of sour cherry pomace extracted at optimum conditions (75 min, 40°C and 42.39% ethanol concentration; solid to solvent ratio: 1/15 (m/v)) by conventional extraction and UAE. The highest phenolic and anthocyanin contents were found in conventional extraction, while the highest antioxidant capacity value was found in UAE samples. Total anthocyanin content values for conventional extraction and UAE were determined as 593 ± 0.9 and 546 ± 0.6 mg cyanidin-3-glucoside equivalent/L

extract, respectively. TPC (TPC) was found as 16,320 ± 5.24 mg GAE/L extract for conventional extraction while it was 15,470 ± 7.43 mg GAE/L extract for UAE.

Okur et al., (2019) compared microwave-assisted extraction, high hydrostatic pressure assisted extraction and UAE for the recovery of sour cherry pomace phenolics. According to the results, these technologies increased the recovery of TPC with respect to conventional solvent extraction using 80% methanol as solvent. UAE was performed at 24 kHz /400 W and 20°C with a solid to solvent ratio of 10 g/ 100 mL 80% aqueous methanol for 15 min. TPC was determined as 239.84 ± 2.89 mg GAE/100 g fresh weight where it was 108.36 ± 3.99 mg GAE/100 g fresh weight for conventional extraction (30 min at 50°C).

The differences among the phenolic amounts presented in the literature may be due to differences in the cultivars and also differences in the extraction methods applied and conditions.

1.3 Antioxidant Properties and Mechanisms

During metabolic processes, reactive oxygen species (ROS) like hydrogen peroxides (H_2O_2) and the superoxide radical anions ($O_2^{\bullet -}$) are the promoters in different reactions. ROS are the free radicals (FR) and unstable molecules. FR can be produced by exogenous and endogenous factors in human body. Intoxication, solar radiation are the examples of exogenous factor. FR are also produced during course of a disease. Excess ROS in a biological systems cause cardiovascular disease and cancer (Santos-Sánchez et al., 2019)

Antioxidants provide inactivation of ROS to control the damage in biological systems. Gordon (1990) classified antioxidants into two class. These are primary (breaking the chain reaction, free radical scavengers) and secondary or preventive antioxidants. Secondary antioxidants can be responsible for deactivation of metals,

lipid hydroperoxide inhibition, regeneration of primary antioxidants and elimination of single oxygen.

Several methods were developed and modified to evaluate antioxidant properties and effectiveness of foods. In literature, antioxidant activity and antioxidant capacity are often confused. Antioxidant activity is related to the rate constant of the reaction between an antioxidant and oxidant. Antioxidant capacity refers to amount of free radicals captured by antioxidants (MacDonald-Wicks et al., 2006). Antioxidant activity cannot be measured using simple chemical reactions in a test tube alone.

The methods for the estimation antioxidant capacity or antioxidant activity are classified into two categories: hydrogen atom transfer (HAT) and single atom transfer (SET) methods (Francenia Santos-sánchez et al., 2019).

Phenolic compounds are antioxidants and can reduce or inhibit free radicals. There are so many studies about determination of antioxidant properties of phenolic compounds in the literature. Polyphenols most likely provides HAT such as gallic acid while kaempferol and resveratrol were the phenolic compounds which were better able to SET (Leopoldini et al., 2004). One method is not reliable for the determination of antioxidant activity/capacity for phenolic compounds due to the differences in their chemical structures and antioxidant effectiveness that cannot be easily classified as HAT and SET mechanisms. SET assays shows the antioxidant's reducing capacity that antioxidant gives an electron to the radical to stabilize it, while HAT assays quantify hydrogen atom donating capacity.

Different assays have been developed to measure antioxidant capacities of plant extracts or food samples. To evaluate the results of these assays, the determination of TPC is also important that the well-known one is Folin-Ciocalteu assay.

1.4 Determination of Total Phenolic Content: Folin–Ciocalteu reducing capacity assay

One of the most important parameters, related to antioxidant capacity determination is the estimation of TPC. The Folin-Ciocalteu (FC) assay is very common method to determine TPC (Shahidi & Zhong, 2015). Initially, this method was used for the analysis of protein by Folin and Ciocalteu (1927) due to the activity of reagent toward tyrosine containing a phenol group and then improved by Singleton et al. (1999) for analysis of phenolic components in wine.

In FC method, phenolic compounds reduce the FC reagent under alkaline condition. Although, chemical nature of FC reagent is not known clearly yet, it is admitted that reagent includes phosphomolybdic/phosphotungstic acid complexes which are reduced by phenolic compounds and other species. Electron transfer from reducing species to molybdenum center (Mo^{+5} to Mo^{+4}) in complex forms blue colored complex which can be detected by spectrophotometer at 750-765 nm (Singleton & Rossi, 1965). Therefore, FC assay is associated with the antioxidant reducing power. Gallic acid is generally used as a standard for calculations and TPC is generally expressed as gallic acid equivalent, GAE (Shaidi & Zhong, 2015). Furthermore, tannic acid, catechin, caffeic acid, chlorogenic acid, vanillic acid and ferulic equivalents were also used to express TPC (Gülçin, 2012). Reaction of Folin’s regent with phenolic compound is given below

$$Na_2WO_4 / Na_2MoO_4 + \text{Phenol} \longrightarrow (\text{Phenol} - MoW_{11}O_{40})^{4-}, \quad (1.1)$$

$$Mo^{5+}_{(Yellow)} + e^- \longrightarrow Mo^{+4}_{(Blue)} \quad (1.2)$$

where Na_2WO_4 is sodium molybdate, Na_2MoO_4 is sodium tungstic and Mo is molybdenum center (Gülçin, 2020)

FC method is simple, reproducible and robust. However, it has several disadvantages like pH, temperature and reaction time sensitivity (Karadağ et al, 2009; Shahidi & Zhong, 2015). During the assay, the reaction between phenolic compounds and the Folin−Ciocalteu reagent takes place at a pH of 10, which is obtained by the addition of sodium carbonate. Under those basic conditions, dissociation of a phenolic proton leads to the formation of a phenolate ion, which is capable of reducing the Folin−Ciocalteu reagent. Since most phenolic compounds are in dissociated form (as conjugate bases, mainly phenolate anions) at the working pH of the assay. Singleton et al. (1999) suggested carrying the assay at room temperature and not exceeding the 3% concentration of the Na_2CO_3 in the reactional mixture. Moreover, it was suggested that the alcohol concentration must be not higher 1% in the reaction mixture, since it can cause the formation of precipitating fine solids due to the interfering effect of methanol. This can be prevented with decreasing methanol concentration and using Na_2CO_3 solution at 5% instead of 20% is used. Carmona-Hernandez et al (2021) studied the effect of assay reagent volume, time of reaction, light exposure, alkali concentration, and temperature on FC assay for the estimation of polyphenol quantitation in Colombian passion fruits. A concentration of 3.5% sodium carbonate was the most appropriate concentration of alkali to react with the FC reagent. Lower alkali concentration yielded lower TPC values, and higher concentrations of the alkali caused turbidity and affecting the spectrophotometric readings. During the assay, light protection is important and there is no requirement of heating. They reported that 90 min is a suitable reaction time at room temperature

In addition, FC reagent doesn't only give a reaction with polyphenols, many non-phenolic organic substances (e.g. ascorbic acid, certain amino-acids, glucose) are reactive with FC reagent that gives overestimated results. Different procedures can be used to reduce the response of interfering compounds such as the partial purification of phenolic compounds by using purification columns (Apak et al 2016; Pico et al., 2020), the calculation of a corrected phenolic content by subtracting the interfering compound reducing capacity from the phenolic content

measured (Lopez-Froilan et al., 2018) and the use of oxidative agents to oxidize interfering compounds prior to assay as an example ascorbate oxidase/H_2O_2 (Sánchez-Rangel et al.,2013).

Furthermore, employment toxic solvents like methanol were other limitations in FC method. Modifications were studied on FC method by Pereira et al. (2018). In this way, alternative solvents like ethanol and water can be used instead of methanol solvent. According to the study, %40 methanol, %40 ethanol and water could be used as solvent by using suitable calibration curves. They proved that there are no significant differences between the results obtained for gallic acid at different concentrations.

Due to their reactivity toward other nonphenolic reducing compounds, the FC assay were also suggested as candidates for measuring the antioxidant capacity of a sample rather than estimating

1.5 Determination of Antioxidant Capacity

Several antioxidant methods have been developed and modified to evaluate antioxidant capacity of different biological samples. Capacity measurements were first used for chemistry and modified and adapted to biology, medicine and nutrition (Floegel et al,2011). Some of the assays for determination of antioxidant capacity are *1,1-Diphenyl-2-picrylhydrazyl* ($DPPH^•$) radical scavenging assay, 2*,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate)* ($ABTS^{•+}$) scavenging assay, ferric reducing antioxidant power (FRAP) assay and cupric ion reducing antioxidant capacity (CUPRAC) assay

1.5.1 $DPPH^•$ Radical Scavenging Assay

$DPPH^•$ radical scavenging assay was suggested as the first approach for antioxidant evaluation of proton donating antioxidants such as phenolic compounds (Brand-

Williams et al.,1995). The purple colored DPPH$^{\bullet}$ in methanol is reduced to the corresponding stable molecule hydrazine (yellow colored) when it reacted with hydrogen donors and measured at 517 nm. Antioxidants can act as radical scavenger by hydrogen donating mechanism or by electron donating mechanism. In the assay, the radical is neutralized by accepting either a hydrogen atom (Figure 1.2) or an electron and it is converted into a reduced form ($DPPH_2$ or DPPH) at the end of the reaction ((Foti et al, 2004; Gülçin 2020)

HAT mechanism: $DPPH^{\bullet} + AH \rightarrow DPPH_2 + A^{\bullet}$ (1.3)

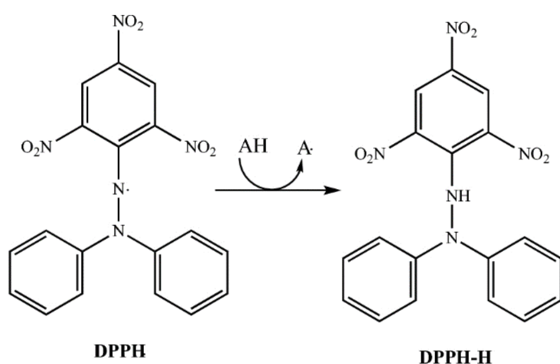
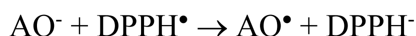


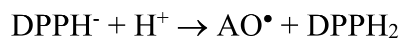
Figure 1.2 DPPH$^{\bullet}$ scavenging mechanisms by an antioxidant (AH) (Gülçin, 2020).

SET mechanism is as follows (1.4)

$$AOH \Leftrightarrow AO^{-} + H^{+}$$



$$AO^{-} + DPPH^{\bullet} \rightarrow AO^{\bullet} + DPPH^{-}$$



$$DPPH^{-} + H^{+} \rightarrow AO^{\bullet} + DPPH_2$$

DPPH$^{\bullet}$ radical is one of the few stable radicals due to the delocalization of the radical in aromatic rings. DPPH assay has been applied to determine antioxidant capacity of food and plant extracts, by using standards such as ascorbic acid, butylated hydroxyl toluene, α-tocopherol, butylated hydroxyl anisole, gallic acid

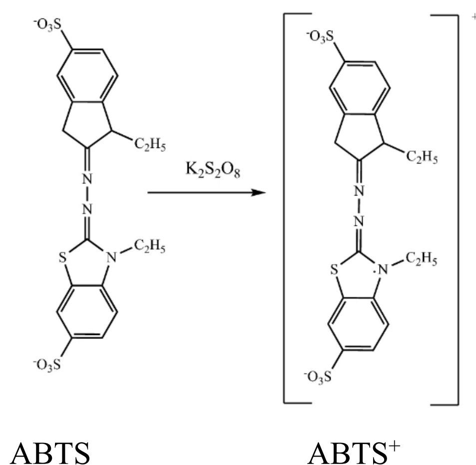
and trolox. But, the antioxidant capacity is generally expressed as percent scavenging capacity or EC_{50} value that means the concentration of antioxidant required to decrease 50% of initial $DPPH^{\bullet}$ concentration. This assay often presents inconsistencies in the literature due to the determination of EC_{50} that depends the initial concentration of $DPPH^{\bullet}$ used and also the mistake in the determination of the percentage of scavenged $DPPH^{\bullet}$ radical, due to the missing measure of the absorbance of the molecular DPPH formed in the reaction. Studies have shown that the scavenging capacity and antioxidant concentration are not linear; so, each sample would need its own calibration curve. The most frequently used reactions times are 30 min, 60 min or the time to reach a plateau (Brand-Williams et al., 1995; Sánchez-Moreno, 2003). Because the radical is stable for several hours, the reaction time must be selected as the time for the reaction of the slowest reacting compound to reach a plateau and can be applied for the faster reacting compounds.

One limitation of this assay is the solubility of $DPPH^{\bullet}$ radical only in organic solvents. Another limitation of this assay was reported as the overlapped spectra of anthocyanins. The absorbtion of anthocyanins is maximum at the same wavelength range of the assay and this cause interference with the results reported (Shaidi & Zhong, 2015). But, this problem can be eliminated by using sufficient dilutions. The main problem reported in the literature is the great variation in the DPPH assay protocol applied and also the insufficient description of the methodology. Therefore, comparison the results with other works may not be impossible.

1.5.2 $ABTS^{\bullet+}$ Radical Scavenging Assay

ABTS is oxidized by oxidants to $ABTS^{\bullet+}$ radical cation which is intensely colored. For this assay, the antioxidant capacity is measured as the ability of antioxidant to decrease the color intensity reacting directly with $ABTS^{\bullet+}$ radical cation (regeneration of stable ABTS). Different oxidants can be used for the production

of $ABTS^{•+}$ radical cations. Potassium persulfate ($K_2S_2O_8$) is generally preferred as shown in Figure 1.3



ABTS ABTS$^+$

Figure 1.3 Oxidation of ABTS with potassium persulfate ($K_2S_2O_8$) and generation of $ABTS^{•+}$ (Gülçin, 2020)

The assay reaction includes HAT and SET mechanisms. According to Prior et al (2005), antioxidant structure and pH of medium are effective for determination of the balance between these two mechanisms. $ABTS^{•+}$ assay can be used in wide pH range that is good opportunities to study the effect of pH on antioxidant mechanisms in food systems. The blue-green colored $ABTS^{•+}$ chromophore absorbs at various wavelengths: 415, 645, 734 and 815 nm. However, 734 nm was the preferred one to eliminate possible interferences and to reduce the sample turbidity is reduced at that wavelength.

The $ABTS^{•+}$ radical is soluble in water and organic solvents, enabling the determination of antioxidant capacity of both hydrophilic and lipophilic compounds. However, $ABTS^{•+}$ radical similar to $DPPH^•$ radical is not found in biological and food systems that makes this radical unrepresentative of

biomolecules. Thermodynamically, a compound that has a redox potential lower than that of $ABTS^{•+}$ may react with the radical (Magalhães et al., 2008). $ABTS^{•+}$ radicals are very reactive if compared with $DPPH^{•}$ radicals. Moreover, this assay is an end point assay similar to $DPPH^{•}$assay that hat is why TEAC value doesn't reflect differences of the reaction rate between antioxidant and oxidants (Huang et al., 2005). Results can be expressed as trolox, ascorbic acid, gallic acid, butylated hydroxyl toluene and butylated hydroxyl anisole equivalents for comparison. If the standard is trolox (water-soluble analog of vitamin E), this assay is also called as trolox equivalent antioxidant capacity (TEAC) assay.

1.5.3 Ferric Reducing Antioxidant Power Assay (FRAP)

FRAP assay was found for measurement of reducing power of plasma. Then, assay was adapted for antioxidant measurements in botanicals (Benzie & Strain, 1996). This assay depends on reduction of ferric 2,4,6-tripyridyl-s-triazine $[Fe^{3+}$-$(TPTZ)]^{3+}$ complex to the blue colored ferrous $[Fe^{2+}$ -$TPTZ]^{2+}$ complex by antioxidant in acidic media (Figure 1.4). Color measurement is performed at 593 nm and results can be expressed as micromolor Fe^{2+} equivalent or according to antioxidant standard. FRAP has totally electron transfer mechanism rather than mixed SET and HAT mechanisms (Prior et al., 2005). Acidic condition at a pH of 3.6 that is adjusted for iron solubility causes decreasing of the ionization potential that increases the redox potential, causing a shift in the dominant reaction mechanism (Simic & Jovanovic, 1994; Hagerman et al., 1998).

FRAP assay is inexpensive and simple, but it has some drawbacks. In FRAP assay, absorption change rate during measurements are different for different phenolic compounds. Prior et al. (2005) claimed that single absorption end point was not enough to present a completed reaction for all antioxidants due to different reactivity. Pulido et al. (2003) claimed that dietary polyphenols such as caffeic acid, ferulic acid, quercetin and tannic acid that reacted more slowly and require longer reaction time for total quantification even after several hours.

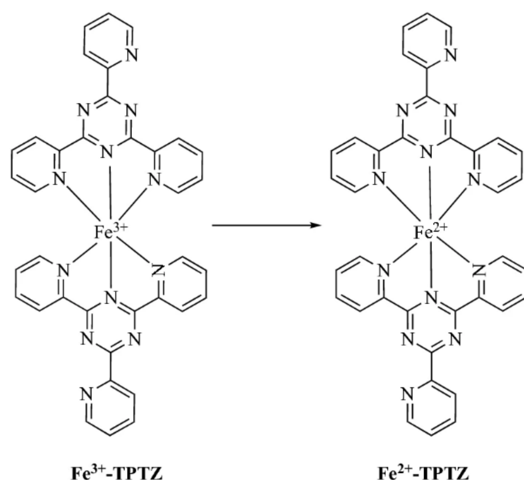


Figure 1.4 [Fe^{3}-$(TPTZ)_2$]$^{3+}$ [Fe^{3+}-$(TPTZ)_2$]$^{2+}$ reduction reaction of FRAP assay (Gülçin, 2020)

This assay cannot measure the antioxidant capacity of certain antioxidants which can react with Fe^{2+} and SH group containing antioxidants. The reducing ability of thiols and carotenoids will not be determined. Generally, FRAP assay had poor correlation with other antioxidant capacity assays and suggested that this assay could be used in combination with other methods to show dominant mechanisms for different antioxidants (Prior et al., 2005).

1.5.4 Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay

This assay was developed and used by Apak et al. (2006). The CUPRAC assay is a copper reduction assay that was improved as a variant of FRAP assay. It is based on conversion cupric ion (Cu^{2+}) to cuprous (Cu^{+}) and measures the reducing power of antioxidant. Ligand is used for formation of copper-ligand complex which is similar to FRAP assay but neocuproine (2,9-dimethyl-1,10-phenanthroline) is chosen as a ligand instead of TPTZ in FRAP assay. In CUPRAC assay, liberated

protons can be buffered by concentrated acetate buffer. Antioxidants reduce Cu^{2+} - neocuproine complex to Cu^{+} - neocuproine that is a chromophore with maximum absorption at 450 nm (Apak et al., 2004). The original CUPRAC assay has been modified like other assays to be used in diverse applications. For example, acetone/water medium with the aid of methyl-β- cyclodextrin has been employed for simultaneous determination of hydrophilic and lipophilic antioxidants (Özyürek et al., 2008)

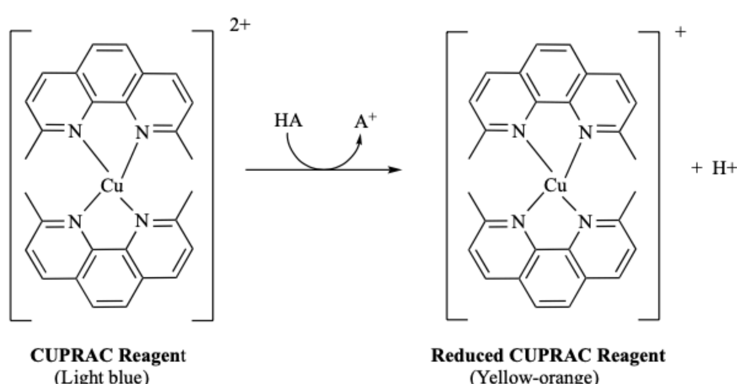


Figure 1.5 CUPRAC mechanism. HA: antioxidant molecule, A+ oxidized anti-oxidant molecule (Gülçin, 2020)

CUPRAC reagent provides a good linear absorbance-concentration curve because of being more stable and accessible than chromogenic radicals. Redox reactions are performed at pH 7 and very close to physiological pH. Thiol type antioxidants like glutathione and non-protein thiol can be measured by using CUPRAC test in contrast to FRAP assay (Gülçin & Daştan, 2007). CUPRAC assay is lower redox potential than Folin's and ferric ion-based reagent so it is very selective. Moreover, most of the antioxidant can be oxidized easily but some of the compounds which are not classified as a true antioxidant like simple sugars and citric acid are not

oxidized with CUPRAC reagent (Apak et al, 2005). CUPRAC assay is one of the most widely used antioxidant capacity assays

1.6 Objective of Study

Fruits and vegetables and also their processed products are important sources of antioxidants for human health. Therefore, there is an increasing interest and research related to antioxidants. Higher production rate of processed fruit and vegetable products provides an important amount of waste such as leaves, seeds, peels and pomace. Utilization of plant based wastes is important because of the presence of bioactive compounds such as phenolic compounds. The characterization and the recovery of phenolic compounds are important for the production of value- added products.

In literature, different studies focused on phenolics and the determination of their antioxidant capacities. However, the assays used for antioxidant capacity measurement not having a clearly demonstrated protocol, give rise to inconsistent uncomparable results, inappropriate applications and interpretations, and improper specification of antioxidant capacity and antioxidant activity.

In this study, different antioxidant assays were used to determine the antioxidant capacity of the selected apple, sour cherry and red grape pomaces. They are direct free radical scavenging assays DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl), and ABTS$^{\bullet}$ (2,20- azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) assays and also reduction capacity assays including cupric ion reducing antioxidant capacity (CUPRAC) and ferric reducing antioxidant power (FRAP) assays. Bioactive compounds of fruits have different characteristics in term of polarity and biological activities. In addition, antioxidant assays differ in terms of their assay mechanisms. Therefore, for assessment of antioxidant potential of different fruit pomace extracts, a single antioxidant assay may not be sufficient for characterization.

The objective of the study is (i) to analyze antioxidant capacity by appropriate applications and interpretations, (ii) to determine the correlation between antioxidant capacities obtained with different assay, (iii) to find the correlation between antioxidant capacity and total phenolic content

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Sour Cherry (*Prunus cerasus* L.; Afyon region), apple (*Malus Domestica* Golden Delicious) and red grape (*Vitis Vinifera* L.;Turkish grape variety: Kalecik Karası) were purchased from local market in Turkey. Fruit pomaces were produced by cold pressing at the fruit juice production pilot plant of Ankara University. The pomace remaining after pressing was mixed well, placed in LDPE bags (30 x 45 cm) and stored at -18ºC.

Gallic acid, methanol, Folin-Ciocalteu reagent (2N) , DPPH$^{\bullet}$, ABTS, sodium acetate, TPTZ, neocuproine were purchased from Sigma -Aldrich Chemie GmbH (Steinheim, Germany). Ammonium acetate, $FeCl_3.6H_2O$, sodium carbonate, potassium persulfate, acetic acid, HCl were purchased from Merck (Darmstadt, Germany). $CuCl_2.2H_2O$ was purchased from Carlo-Erba (Val-de Reuil, France). Ethanol (96%) was purchased from ITK (İstanbul Teknik Kimya A.Ş., Turkey). Distilled water was used during the experiments.

2.2 Methods

2.2.1 Pomace Drying

Pomace samples were dried in a tray dryer (EKSİS Industrial Dryer Systems, Isparta, Turkey) at 60°C and 5% *RH* with the air velocity of 1 m/s, for approximately 8 h. Dried pomaces were ground by using a blender (NSF,

Blendersi, Hamilton Beach Brands Inc. US). The particles that passed through the sieve (certified sieves, Fritsch,Germany) an opening size of 1 mm were used for extractions. Moisture content of the dried sour cherry, grape and apple pomaces were determined as 6.81 ± 0.374 %, 4.46 ± 0.142 % and 2.77 ± 0.069 % (n=3), respectively. The dried and ground samples were also kept at 4°C.

2.2.2 Ultrasound-assisted Extraction(UAE)

Dry pomace samples were extracted with distilled water, 50% (v/v) aqueous ethanol and ethanol by using a high power ultrasonic processor (UP 400 S, 24 kHz, Dr. Hielscher GmbH, Stuttgart, Germany) with H14 probe (105 W/cm^2, 125 μm) at 0.5 pulse control mode (cycle: power discharge 0.5 seconds, pause 0.5 seconds) for 5 min. The ratio of dry pomace to solvent was 5 g : 50 mL for the extraction of phenolic compounds. The extraction process was performed in an ice-bath. The temperatures of extracts were in the range of 35 - 40°C at the end of sonication. Then the extracts were hold at room temperature for 24 h for additional extraction. After extraction, the solutions were filtered through a filter cloth and then centrifuged at 7000xg and 4°C for 5 min (Nüve NF 1200R, Ankara, Turkey). The supernatant was stored at 4°C for analysis. The extractions were two times replicated.

2.2.3 Determination of Total Phenolic Content (TPC)

TPCs of extracts were determined according to Folin-Ciocalteu method of Singleton and Rossi (1965) with slight modifications. 10 mL Folin-Ciocalteu reagent (2 N) was diluted with 90 mL water. %7.5 Na_2CO_3 solution was prepared in water. 1 mL extract was diluted with 9 mL water before the assay application. Additional dilutions were done, if required, to adjust the sample A_{760} values to the calibration line absorbance range.

5 mL diluted Folin-Ciocalteu reagent was placed into a test tube and 1mL diluted extract was added. After mixing, the mixture was hold for 5 minutes. Then 4 mL Na_2CO_3 solution was added. The reactional mixture in the tube were kept in the dark place for 90 min. In order to find equilibrium reaction time, 0.1 and 0.5 mg/mL GA solutions in different solvents were tested for 2 h reaction period and it was observed that the reaction was stable after 60 minutes at room temperature. The color of the samples was turned from yellow to blue. Absorbance of the samples (A_{760}) were measured at 760 nm (UV/VIS spectrophotometer, SHIMADZU UV–1700 Pharma Spec, Kyoto Japan). Extraction solvents were used as blanks.

For the calibration line, gallic acid was used as standard in a concentration range of 0.1- 0.5 mg/mL by using the same procedure (Figure 2.1). Calibration lines were obtained for extraction solvents which are water, ethanol and 50% aqueous ethanol used. TPC of apple, sour cherry and grape pomace extract was expressed as gallic acid equivalent (mg GAE/mL extract).

2.2.4 Determination of Antioxidant Capacity (AC)

Ferric reducing ability of power (FRAP), cupric reducing antioxidant capacity (CUPRAC), 2,2-Diphenyl-1-picryhydrazyl radical (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays were used for the estimation of AC of the apple, sour cherry and grape pomace extracts. During the assays, absorbance values of the samples were measured in 10 min interval for 120 min at the assay wavelength to determine the reaction time to reach plateau (equilibrium).

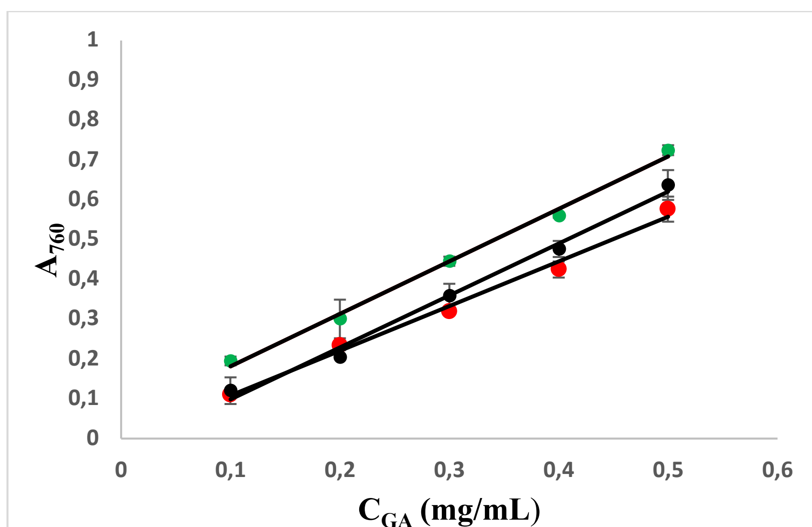


Figure 2.1 Calibration lines of Folin-Ciocalteu method for gallic acid (GA) standard in ethanol, 50% aqueous ethanol and water: Data were presented as mean with standard deviation of three measurements (n=3)

- ethanol $A_{760} = (1.305)\ C_{GA} - 0.032$ $R^2 = 0.99$
- 50 % ethanol $A_{760} = (1.319)\ C_{GA} + 0.050$ $R^2 = 0.99$
- water $A_{760} = (1.123)\ C_{GA} - 0.004$ $R^2 = 0.99$

2.2.4.1 Ferric Reducing Antioxidant Power (FRAP) Assay

Frap assay was performed according to Benzie and Strain (1996) with slight modifications. The stock solutions were 300 mM acetate buffer solution (pH 3.6), 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution and 20 mM $FeCl_3.6H_2O$ (iron(III) chloride hexahydrate) solution.

For the preparation acetate buffer solution, 3.1 g sodium acetate ($NaCH_3COO$) was added to 16 mL acetic acid (CH_3COOH) and then diluted to 1 L with water to prepare 300 mM acetate buffer. For the preparation of 10 mM TPTZ solution, 0.031g TPTZ was dissolved in 10 mL of 40 mM HCl. For the preparation 20 mM $FeCl_3.6H_2O$ solution, 0.054g $FeCl_3.6H_2O$ was dissolved in 10 mL water

25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3.6H_2O$ solution were mixed to prepare fresh working solution (FRAP solution). 0.1 mL fruit extract was added to 2.9 mL FRAP solution. The yellow-orange color was changed to intense blue color. 0.1 mL extraction solvent was mixed with 2.9 mL FRAP solution to prepare control sample. The reactional mixtures were kept in the dark place for 90 min at room temperature. Control samples were prepared by adding 1 mL extraction solvent instead of extracts. The absorbance of the samples (A_{593}) were measured at 593 nm (UV/VIS spectrophotometer, SHIMADZU UV–1700 Pharma Spec, Kyoto Japan). Water was used as blank. Results were expressed as GAE/mL obtained from the gallic acid calibration line in the concentration range of 0.001-0.05 mg GA/mL. The calibration lines were plotted as ΔA_{593} (A_{sample} – $A_{control}$) values vs. the concentration of gallic acids (Figure 2.2). For samples, additional dilution was done if the absorbance value measured was over the linear range of the standard line.

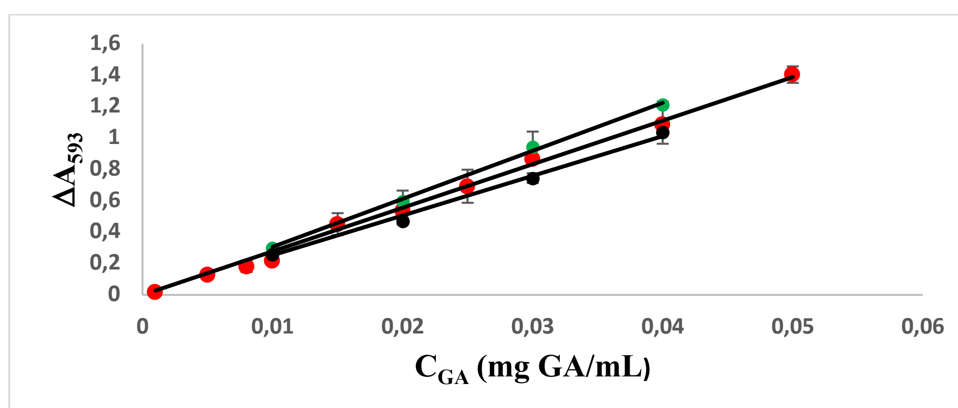


Figure 2.2 Calibration lines of FRAP assay for gallic acid (GA) standard in ethanol, 50% aqueous ethanol and water: Data were presented as mean with standard deviation of three measurements (n=3)

• ethanol $\Delta A_{593} = 27.757\ (C_{GA})$ $R^2 = 0.99$

• 50 % ethanol $\Delta A_{593} = 25.293\ (C_{GA})$ $R^2 = 0.99$

• water $\Delta A_{593} = 30.602\ (C_{GA})$ $R^2 = 0.99$

2.2.4.2 Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay

CUPRAC assay was performed according to the method of Apak et al., (2004) with slight modifications. The stock solutions were 10 mM $CuCl_2$. $2H_2O$ solution prepared in water, 7.5 mM neocuproine solution prepared in methanol and 1 M ammonium acetate ($NH_4CH_3CO_2$) buffer (pH=7).

After preparation of the solutions, 1mL of $CuCl_2.2H_2O$ solution, 1 mL of neocuproine solution and 1mL of ammonium acetate buffer solution were mixed a test tube and then 1mL of extract was added to this mixture. The reactional mixtures were kept in the dark place for 90 min at room temperature. The yellow color was observed which was caused by reduction of Copper(II) ion. Control samples were prepared by adding 1 mL extraction solvent instead of extracts. Ammonium acetate buffer was used as a blank sample. Absorbance values of the samples were measured at 450 nm. Results were expressed as GAE/mL obtained from the gallic acid calibration line in the concentration range of 0.001-0.03 mg GA/mL. The calibration lines were obtained as ΔA_{450} ($A_{sample} - A_{control}$) values vs. the concentration of gallic acid standard concentration (Figure 2.3). For samples, additional dilution was done if the absorbance value measured was over the linear range of the standard line.

2.2.4.3 DPPH Free Radical Scavenging Assay

$DPPH^{\bullet}$ radical scavenging assay was performed according to the method of Brand-Williams et al., 1995 and Kumaran et al., 2006 some modifications. 3.6 mL DPPH solution(0.1mM) was mixed with 0.4 mL extract and then the mixture was kept in the dark room at room temperature for 90 min. Control sample was prepared by adding 0.4 mL methanol to 3.6 mL $DPPH^{\bullet}$ solution. Methanol was used as a blank and absorbance values of samples (A_{515}) were measured at 515 nm (UV/VIS spectrophotometer, SHIMADZU UV–1700 Pharma Spec, Kyoto Japan) after a reaction time of 90 min.. The purple color of the samples was changed to yellowish

with the existence of antioxidant. Figure 2.4 shows the calibration curve obtained with GA in methanol (0.001 – 0.05 mg GA/mL).

Due to this non-linearity, extracts were diluted in methanol (10% - 75 %, v/v) to obtain sample calibration curves. Additional dilution were done with methanol if required. Percentage scavenging capacity was calculated from equation 2.1.

$$SC\ (\%) = \frac{A_{control} - A_{sample}}{A_{conttrol}} x100 \tag{2.1}$$

DPPH$^{\bullet}$ SC and antioxidant standard concentration relationship is not linear. In the literature, for this purpose, the predictive mathematical models such as Logarithmic model, Power model, Logistic equation and Hill equation, or comparative approach based on various statistical regression models were used for different standards and plant extracts (Carmona-Jiménez et al. 2014; Suriyatem et al., 2017; Sridhara and Charles, 2019). Exponential distribution model (equation 2.2) and logarithmic model (equation 2.3) were used to present the change of SC with respect to sample TPC:

$$\frac{\mathrm{SC}_{\mathrm{equlibrium}} - \mathrm{SC}_{\mathrm{sample}}}{\mathrm{SC}_{\mathrm{equilibrium}}} = \mathrm{e}^{-\alpha[C_s]} \tag{2.2}$$

$$SC = aln[C_s] + b \tag{2.3}$$

where C_s is the concentration of standard: GA (mg GA/ml) or extract TPC (mg GAE/ml)

Percentage of scavenging capacity was also expressed using effective concentration (EC) values. EC_{50} and EC_{20} are reported as the concentration of antioxidant required to decrease the initial concentration of the radical by 50 and 20%, respectively.

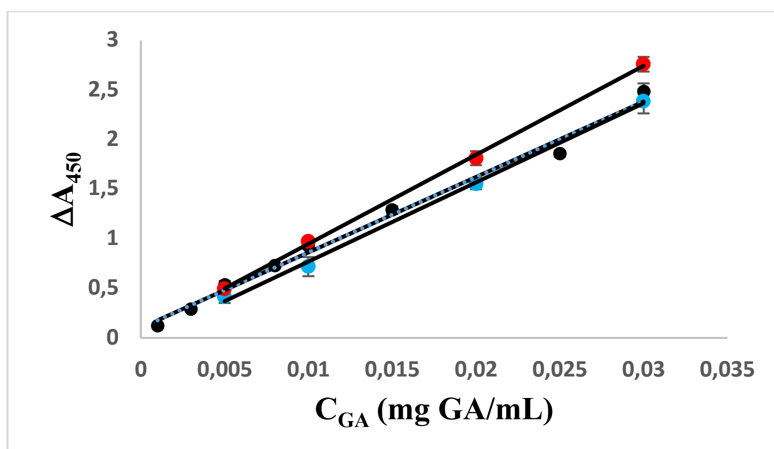


Figure 2.3 Calibration lines of CUPRAC assay for gallic acid (GA) standard in ethanol, 50% aqueous ethanol and water: Data were presented as mean with standard deviation of three measurements (n=3)

- ethanol $\Delta A_{450} = 76.063\ (C_{GA}) + 0.1017$ $R^2 = 0.99$
- %50 ethanol $\Delta A_{450} = 89.810\ (C_{GA}) + 0.0506$ $R^2 = 0.99$
- water $\Delta A_{450} = 79.087\ (C_{GA}) + 0.0291$ $R^2 = 0.99$

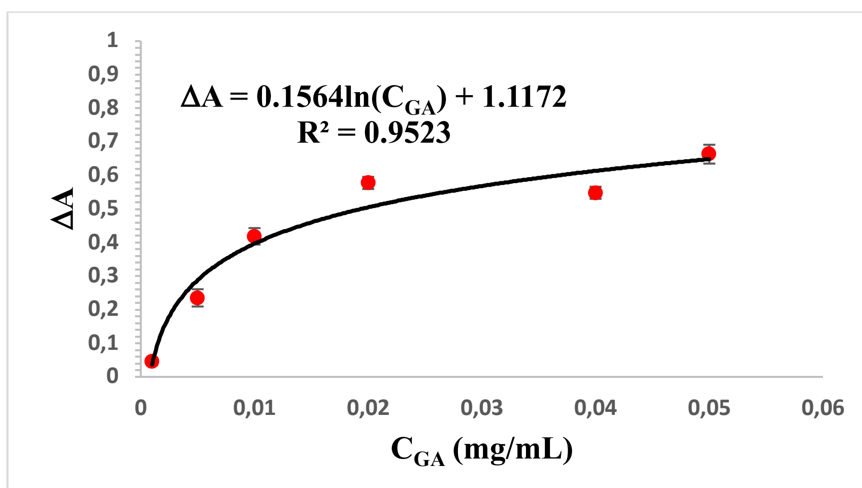


Figure 2.4 Calibration curve of DPPH• assay for gallic acid (GA) standard in methanol: Data were presented as mean with standard deviation of three measurements (n=3)

2.2.4.4 ABTS Radical Scavenging Assay

This radical scavenging assay was performed according the method of Miller and Rice-Evans (1997) and Re et al., (1999) with some modifications. 38.3 mg ABTS was dissolved in 10 mL water to obtain 7mM $ABTS^{•+}$ solution. 6.6 mg potassium persulfate ($K_2S_2O_8$) was dissolved in 10 mL distilled water to obtain 2.45 mM solution. $ABTS^{•+}$ and potassium persulfate solutions were mixed (1:1 v/v) and kept in a dark for 12-16 hour to obtain radicalization ($ABTS^{•+}$ formation). Then 2 mL of this $ABTS^{•+}$ solution was completed to 80 mL by adding methanol to adjust the absorbance (A_{734}) of the solution to 0.700 ± 0.05 at 734 nm. For the assay, 2 mL $ABTS^{•+}$ was mixed with 1 mL sample. After 90 min, the absorbance values of the samples were at 734 nm (UV/VIS spectrophotometer, SHIMADZU UV–1700 Pharma Spec, Kyoto Japan). Control sample was prepared by adding 1mL methanol to 2 mL $ABTS^{•}$ mixture. Methanol was used as blank sample.

The calibration curves for extracts in methanol (dilution: 10% - 75 %, v/v) were obtained for a reaction time of 90 min. Similar to $DPPH^{•}$ assay, percentage scavenging capacity was calculated from equation 2.1. Percentage of scavenging capacity was also expressed using EC_{50} and EC_{20}. A mathematical model similar to the exponential distribution model (equation 2.2) and logarithmic model (equation 2.3) were used to present the change of SC with respect to TPC. Additional dilution were done with methanol if required.

2.3 Statistical Analyses

Each analysis was performed in triplicate and the results were reported as mean value ± standard deviation. The data were analyzed by Minitab Statistical Software (19. 2020. 2.0 version, Minitab Ltd., UK) by using oneway analysis of variance (ANOVA) at 0.05 significance level, and Tukey's New Multiple Range Test was applied to analyze the results of the experimental data. The Pearson test was

applied to identify the differences between TPC and AC tests ($p<0.05$). This statistical results presented in Appendix

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Total Phenolic content (TPC)

For the estimation of TPCs, Folin−Ciocalteu assay was used by using calibration lines obtained by dissolving GA in water, 50% aqueous ethanol and ethanol (Figure 2.1). Bastola et al. (2017) indicated that selection of a suitable standard leads to more accurate estimation of phenolics and GA was found to be the best single standard among the phenolic standards used in their study. The solvent affects the performance of the assay due to phenolic compound solubility and precipitate formation. Water solvent provided a decreased absorbance response. Similar results were obtained in the study of Pereira et al., (2018) that gallic acid stock solutions (0.1 mg/mL) were prepared in water, 40% aqueous methanol (v/v) or 40% aqueous ethanol (v/v). The presence of ethanol affects the slope of the calibration lines. During the assay, the reaction between phenolic compounds and the Folin−Ciocalteu reagent takes place at a pH of 10, which is reached by adding Na_2CO_3. Since most phenolic compounds are in dissociated form (as conjugate bases, mainly phenolate anions) at the working pH of the assay, they can be more easily oxidized with the Folin−Ciocalteu reagent. Singleton et al. (1999) reported that the formation of fine solids depended on the initial carbonate concentration. Additionally, to obtain reproducible results, it was suggested that alcoholic concentration in the final reaction mixture was not exceeding the 1% as in this study. The effect of initial carbonate concentration in the presence of methanol was studied by Cicco and Lattanzio (2011). They reported that Na_2CO_3 concentration in the presence of methanol, was a critical parameter since it could modify the formation of precipitating fine solids. The formation of particles delayed with decreasing methanol concentration. Carmona-Hernandez et al. (2021) reported

lower alkali concentration yielded lower TPC values, and higher concentrations of the alkali provided a more suitable medium for the formation of phenolate ions but generating turbidity that affected the spectrophotometric readings. In our study, any precipitation was not observed for the selected assay conditions.

Phenolic contents and composition of the selected pomaces are different from each other. The polyphenols of Golden Delicious apple pomace are mainly flavonoids followed by phenolic acids. The sour cherry pomace contains mainly anthocyanins and flavonoids. Anthocyanins are the main polyphenolics in red grape pomace. Table 3.1 shows TPCs of apple, sour cherry and grape pomace extracts. According to the results, water provided the highest extraction of phenolic compounds (0.164 ± 0.075a mg GAE/ mL extract) from apple pomace. 50% aqueous ethanol provided the highest extraction of phenolic compounds from sour cherry and grape pomaces. TPC results of sour cherry and grape pomace extracts were found as 0.749 ± 0.046 and 2.803 ± 0.205 mg GAE/mL extract, respectively.

Table 3.1 Total phenolic contents results of apple, sour cherry and grape pomace extracts

	TPC	
Solvent	**mg GAE/mL extract)**	**(mg GAE /g dry pomace)**
	Apple pomace	
Water	$0.164 \pm 0.075^{a*}$	1.636 ± 0.754^{a}
50 % aqueous ethanol	0.075 ± 0.006^{c}	0.750 ± 0.057^{c}
ethanol	0.089 ± 0.002^{b}	0.893 ± 0.025^{b}
	Sour cherry pomace	
Water	0.530 ± 0.027^{b}	5.341 ± 0.255^{b}
50 % aqueous ethanol	0.749 ± 0.046^{a}	7.492 ± 0.462^{a}
ethanol	0.513 ± 0.110^{b}	5.142 ± 1.096^{b}
	Grape pomace	
Water	1.423 ± 0.126^{b}	14.221 ± 1.216^{b}
50 % aqueous ethanol	2.803 ± 0.205^{a}	28.020 ± 2.040^{a}
ethanol	1.280 ± 0.060^{b}	12.822 ± 0.575^{b}

* different letters within the same column shows significance difference ($p \leq 0.05$)

Zhang et al. (2016) reported TPCs of Golden Delicious apple pomace extracts with methanol, ethanol, acetone, ethyl acetate and chloroform. It was varied significantly according to the extraction medium, ranging from 1.62 to 3.05 mg GAE/g powder (1 g of powder /5 mL of solvent ratio in ultrasonic bath at 37 °C for 40 min) that their results were higher than the results obtained in this study. The highest level of TPC was detected in the methanol extract and methanol and ethanol showed insignificant differences for extraction in their study. Rana et al. (2021) determined TPCs of different apple cultivar pomaces in the range of 2.19 ± 0.09 and 4.59 ± 0.47 mg GAE/g dry pomace that it was 2.7 mg GAE/g dry pomace for Golden Delicious apple. In their study, the dry pomace samples were extracted with 70% aqueous methanol (v/v; 1 g powder/20 mL solvent) at 60°C for 30 min. Their results are higher than the results obtained in this study due to use of methanol as solvent that is not preferred for industrial applications. Although safety could still be achieved by the removal of the organic solvents from the final product, producers and consumers prefer products which are not processed using methanol. After the extraction of apple pomace with water, the solid part in the extract was similar to apple puree in our study. This might be due to US induced cell wall disintegration accompanied with intensive disintegration of skin tissues. In the literature, water extraction reported as an opportunity to extract the antioxidants of apple pomace. Candrawinata et al. (2014) studied the effects of extraction time (5-60 min), temperature (20-90°C) and water to fresh pomace ratio (10-120 mL / 5 g) on TPCs and antioxidant capacities of apple pomace (unknown cultivar) aqueous extracts. The optimum extraction condition was determined as water to fresh pomace ratio of 20 mL:1 g at 90°C for 15 min yielding the most polyphenolic compounds (1.148 mg GAE /g fresh pomace). These results indicated that water can be used as a solvent for extracting polyphenolics from apple pomace, however, as compared to the methanol extract (control), the aqueous extracts had lower total phenolic content (63% of methanol extract) and antioxidant capacity (73-80% of methanol extract) that their results was not in accordance with the results obtained in this study that extraction with ethanol provided lower TPC

for Golden Delicious apple pomace. Krasnova I. and Segliòa (2019) were also applied water extraction (200 g of fresh apple pomace/800 mL hot water at an initial 95°C temperature and then macerated for 10 h) for 11 apple cultivars and TPCs was in the range 0.14 – 0.278 mg GAE/ g dry weight and they reported that water extraction could be used for apple pomaces.

Gonelimali et al. (2021) showed that pomace drying conditions affected extraction results. Based on their results, drying apple pomace at 60°C using conventional atmospheric oven provided significantly higher antioxidant capacity. The other aspect to be considered is the possible effect of polyphenol oxidase activity in the pomace. The enzyme is suggested to be optimally activated at 50°C and could potentially lower the total phenolic content and subsequently the antioxidant capacity of the extracts. To stop or slow down this effect, it is necessary to dry the apple pomace immediately upon the production (Kammerer et al., 2014). This enzyme is denatured at approximately 60-70°C. pH also is important for polyphenol oxidase capacity that is stable at pH range of 6 to 8 and is likely to become unstable below pH 4.5 in various fruits, including apples.

The other pomace used in this study was sour cherry pomace. 50 % aqueous ethanol solvent provided the highest TPC in the extract. Yılmaz et al. (2015) investigated the effects of the extraction parameters on TPC for sour cherry pomace. 51 % aqueous ethanol, 75 °C, 12 mL solvent /g solid ratio and 100 min were determined as the optimum extraction parameters during solid-liquid extraction. At this optimum condition, TPC was determined as 14.23± 0.38 mg GAE/g dry pomace that was higher in comparison to our result obtained with 50% aqueous ethanol solvent (7.492 ± 0.462 mg GAE/g dry pomace). In their study, increasing ethanol concentration above the optimum value caused to decrease in TPC values of the extracts due coagulation especialy due to proteins as reported. TPC of pomace extract with water and ethanol solvents were reported as 0.36 and 1.63 mg GAE/g dry pomace at the extraction condition of 1/9 solvent to solid ratio (v/w) and 50 °C for100 min. These values were lower than the results obtained in

this study for water and ethanol solvents. Similarly, according to the study of Simsek et al (2012), the solvent type was significantly effective on phenolic extraction. TPC value of freeze dried sour cheery pomace extract was higher for ethanol:water (1:1 v/v) solvent (13.78 mg GAE /g dry sample), if compared with that of water and ethanol solvents during conventional extraction (30mL /g solvent to solid ratio; 700 W heating power; 6 h). Their results are in accordance with the result obtained from our study that 50 % aqueous ethanol solvent provided the highest TPC in extracts (7.492 ± 0.462 mg GAE/g dry pomace). But their reported TPC value was higher than the TPC value obtained with 50 % aqueous ethanol solvent in this study due to differences in extraction conditions and especially drying technique used. Drying conditions have significant effects on loss of phenolic compounds. Horuz et al. (2017) compared the TPC results of sour cherry by using conventional drying (0.5 m/s air flow rate at 50, 60, and 70°C) and hybrid drying(microwave-convectional drying at 120, 150, and 180W coupled with hot air at 50, 60, and 70°C). Extractions were done in 100 mL ethanol: acetone (70:30 v/v) solvent for 5 g of fresh or dried sample at 37°C for 1 h. TPC of fresh sour cherries was found as 12.343 mg GAE/ g dry solids. However, TPC values of dried sour cherry samples were found in the range of 2.255 – 3.859 mg GAE/g dry solids which means 68.7–81.7% TPC loss during the conventional drying. They claimed that loss of TPC of lower during hybrid drying due to the long exposure to oxygen and thermal deterioration of the phenolics during conventional drying.

Red grapes contain higher amount of phenolics. Aşcı and Göktürk (2021) determined TPCs of different fresh grape varieties (Kalecik Karası, Öküzgözü, Emir, and Narince) in the range of 1.87 ± 0.14 – 3.42 ± 0.15 mg GAE/g fresh weight that the highest TPC was found for Kalecik Karası grapes. Red grape pomace is also a rich source of bioactive compounds. In the study of Baydar et al., (2011), TPCs of Kalecik Karası grape dry defatted seed and skin extracts (Soxhlet extraction; 8 h; acetone: water: acetic acid (90:9.5:0.5) at 60°C) were determined as 526.55 ± 9.97 and 43.75 ± 1.48 mg GAE/ g extract, respectively. In our study, the

highest TPC (28.020 ± 2.040 mg GAE/ g dry pomace) was obtained with 50% aqueous ethanol solvent.

In the literature, the effects of using different solvents and extraction techniques on recovery of phenolic compounds from different varieties of grape pomace during were reported. Hogan et al. (2010) determined TPC of red grape pomace (Cabernet Franc) and white grape pomace (Chardonnay) extracts that were prepared in 80% ethanol (1 g powder /10 mL solvent under overnight shaking). TPC of red and white grape pomaces were found as 30.4 ± 11.0 mg GAE/g dry pomace and 24.5 ± 6.0 mg GAE/g dry pomace. The results were in accordance with the results obtained in this study that the extraction with 50% aqueous ethanol provided the TPC as 28.020 ± 2.040 mg GAE/ g dry pomace. Pintac et al (2018) studied on solvent selection for phenolic extraction of different red and white grape pomaces. They found the TPC of pomace extracts with 80% aqueous ethanol (1 g sample/10mL solvent for 6 h at room temperature) in the range of 16.9 - 64.8 mg GAE/g dried extract or 1.93-4.26 mg GAE/g fresh weight.

Guala et al. (2016) studied the effects of solvent type on phenolic extraction from grape pomace during UAE. Solvent type significantly affected TPC. They found that the yield of total phenolics was decreased in the following order with 50 % aqueous ethanol > 70% aqueous ethanol > 70% aqueous methanol > ethanol > water. These results were in accordance with the results obtained in this study that grape pomace extraction with 50% ethanol provided highest TPC. In our study, there was no significant difference between TPCs of water and ethanol extracts. Another comparative study was performed by Drosou et al. (2015). They studied the effects of solvent type and drying techniques (air drying and accelerated solar drying) on TPC of grape pomace (*Vitis vinifera*) extracts during conventional extraction, UAE and microwave-assisted extraction. Air dried grape pomace provided better extraction of total phenolics than TPCs of accelerated solar drying and undried grape pomaces for all of the extraction techniques used. They also claimed that drying treatment and grinding process affected extraction positively

due to the increase in surface area. UAE with 50% aqueous ethanol solvent (g sample /70 mL solvent; 25 kHz; 300 W; 20°C; 60 min) exhibited higher TPC (438.984 ± 4.034 mg GAE/ g dry extract) than that of UAE with water (50.959 ± 2.917 mg GAE/ g dry extract) for air dried grape pomace (55 ± 2°C; 1.0 m/s air velocity; 24 h). Among different extraction techniques, UAE with 50% aqueous ethanol exhibited the highest TPC. Xu et al. (2015) claimed that increasing the temperature from 40 to 80°C for UAE, had negative effects on phenolic compounds. During our study, the temperatures of extracts were in the range of 35 - 40°C at the end of sonication. Processing time is an important parameter for extraction of phenolics and the time must be selected according to the type of ultrasonic processor. Generally, the maximum extraction time was selected not longer than 30 min for UAE (Casazza et al. 2010; González-Centeno et al. 2015; Dranca & Oroian 2019).

In the literature, for the estimation of phenolic content, Folin−Ciocalteu assay is generally used. Other reducing compounds besides phenolics can reduce Folin−Ciocalteu reagent due to indefinite redox potential (Apak et al. 2016). According to the different studies, the interference of glucose, HMF, furfural, and vitamin B-12 did not significant in phenolic estimation whereas ascorbic acid interfered (Everette et al., 2010; Bastola et al., 2017). During performing the TPC assay, before the addition of the alkali, ascorbic acid rapidly may react with polyphosphotungstate. Therefore, the blue color formation observed under the assay initial acidic condition might be attributed to the ascorbic acid content as reported in the study of Sanchez-Rangel et al. (2013). Sun et al. (2002) reported that ascorbic acid only contributed 0.4 % and 0.76 % of the total antioxidant capacity in apples and peach, respectively. This number is 0 and 3 % for cranberry and strawberry. Isabelle et al. (2010) were reported to possess reducing activity of 0.872 mg GAE/mg AA for acetone/water/acetic acid mix solvent. It was reported as 0.571 mg GAE/mg AA for ethanol in the study of Showkat et al. (2019). For the estimation of TPC of fruits and fruit-based products, Folin−Ciocalteu assay was widely used without ascorbic acid correction in the literature. Also fruit pomaces

have lower vitamin C content, if compared with TPC. As an example, Ciccoritti et al. (2018) reported phenolic and ascorbic acid contents of sour cherry pomace (a local variety, Italy) as 45 ± 1 mg GAE/g dry pomace and 2.5 ± 0.3 mg AA/g dry pomace, respectively. For Kalecik karası grapes, TPC of the 23 clones varied from 3.310 to 3.389 mg GAE / g fresh weight while vitamin C content ranged from 0.14 to 0.165 mg/ g fresh weight (Keskin et al., 2014). The phenolic content of Cabernet Saugvinon and Feteasca Neagra grape pomace extracts in ethanol were reported as 265.21 ± 4.97 and 279.64 ± 4.52 mg GAE/ g dry extract, respectively (Brezoiu et al., 2019). They also measured ascorbic acid contents as 45.54 ± 0.14 and 33.79 ± 0.56 mg /g dry extract for Cabernet Saugvinon and Feteasca Neagra grape pomaces. But, for Golden delicious apple peel, TPC and ascorbic acid content were measured as 5.22 mg GAE/g freeze dried peel and 1.24 mg/ g freeze dried peel that ascorbic content was high. The cultivar Golden Delicious contained the significantly lowest content of total polyphenols in both peel and flesh among15 cultivars.

3.2 Total Antioxidant Capacity

Total antioxidant capacities of the fruit pomaces were determined by using FRAP, CUPRAC, DPPH and ABTS assays

3.2.1 Ferric Ion Reducing Antioxidant Power (FRAP) and Cupric Ion Antioxidant Capacity (CUPRAC) Methods

FRAP analysis detects the reducing capacity of any antioxidant to reduce iron ion. Table 3.2 shows FRAP assay results obtained for the selected pomace extracts. Although the highest TPC was obtained for water extract, the highest FRAP value was obtained for the extraction of apple pomace with 50% aqueous ethanol (0.0307 ± 0.0012 mg GAE/mL extract). For sour cherry and grape pomace extracts, the highest TPC and FRAP values were obtained with 50% aqueous ethanol.

Table 3.2 FRAP results of apple, sour cherry and grape pomace extracts

	Antioxidant Capacity		
Solvent	**mg GAE/mL extract**	**mg GAE/ g dry pomace**	**mg GAE /mg TPC**
		Apple pomace	
Water	$0.0160 \pm 0.0010^{b*}$	0.164 ± 0.006^{b}	0.100 ± 0.003^{b}
50 % aqueous ethanol	0.0307 ± 0.0012^{a}	0.310 ± 0.010^{a}	0.413 ± 0.136^{a}
ethanol	0.0126 ± 0.0012^{c}	0.127 ± 0.011^{c}	0.143 ± 0.012^{c}
		Sour cherry pomace	
Water	0.2110 ± 0.0130^{b}	1.780 ± 0.040^{b}	$0.411 \pm 0,026^{a}$
50 % aqueous ethanol	0.3377 ± 0.0236^{a}	3.377 ± 0.236^{a}	0.448 ± 0.031^{a}
ethanol	0.1780 ± 0.0040^{b}	2.110 ± 0.130^{b}	0.333 ± 0.003^{b}
		Grape pomace	
Water	0.320 ± 0.011^{b}	3.203 ± 0.117^{b}	$0.225 \pm 0,415^{b}$
50 % aqueous ethanol	1.133 ± 0.081^{a}	11.347 ± 0.789^{a}	$0.405 \pm 0,028^{a}$
ethanol	0.259 ± 0.011^{b}	2.588 ± 0.117^{b}	$0.202 \pm 0,009^{b}$

* different letters within the same column shows significance difference ($p \leq 0.05$)

Table 3.3 CUPRAC results of apple, sour cherry and grape pomace extracts

	Antioxidant Capacity		
Solvent	**mg GAE/mL extract**	**mg GAE/ g dry pomace**	**mg GAE /mg TPC**
		Apple pomace	
Water	$0.063 \pm 0.008^{a*}$	0.632 ± 0.091^{a}	0.385 ± 0.053^{b}
50 % aqueous ethanol	0.057 ± 0.002^{a}	0.567 ± 0.023^{a}	0.760 ± 0.027^{a}
ethanol	0.033 ± 0.004^{b}	0.330 ± 0.035^{b}	0.371 ± 0.038^{b}
		Sour cherry pomace	
Water	0.553 ± 0.032^{a}	5.527 ± 0.314^{a}	1.007 ± 0.061^{a}
50 % aqueous ethanol	0.573 ± 0.029^{a}	5.732 ± 0.296^{a}	0.761 ± 0.039^{b}
ethanol	0.374 ± 0.083^{b}	3.738 ± 0.829^{b}	0.705 ± 0.157^{b}
		Grape pomace	
Water	0.915 ± 0.029^{b}	9.153 ± 0.282^{b}	0.643 ± 0.020^{c}
50 % aqueous ethanol	1.271 ± 0.070^{a}	12.385 ± 0.159^{a}	0.453 ± 0.025^{a}
ethanol	0.699 ± 0.033^{c}	6.996 ± 0.331^{c}	0.547 ± 0.027^{b}

* different letters within the same column shows significance difference ($p \leq 0.05$)

The principle of CUPRAC method is similar to that of the FRAP method that ferric ion is replaced by cupric ion. Table 3.3 shows CUPRAC assay results obtained for the selected pomace extracts. For apple and sour cherry pomace extracts, no significant difference was detected between extracts obtained with water and 50% aqueous ethanol. For grape pomace extract, the highest CUPRAC value (1.271 ±

0.070 mg GAE/mL extract) was obtained by extraction with 50% aqueous. For these methods, generally, the sour cherry pomace extracts provided higher GAE to TPC ratio if compared with that of apple and grape pomace extracts.

FRAP and CUPRAC calibration curves were perfectly linear for the selected concentration ranges Figue 2.2 and 2.3) for different solvent. GA was used as standard for these assays. For the selected solvents, relative standard deviation was 9.53 and 8.847 % for FRAP and CUPRAC calibrations lines, respectively. Relatively independent of solvent effects in alcohol–water mixtures of varying composition were observed for a wide trolox concentration range and linear calibration equations for CUPRAC method by Özyürek et al., (2011). Although direct comparison of the antioxidant capacity assay results obtained in this study with results reported in literature, may not possible due to differences in assays applied or standards used to report the results, some are presented here for comparison. Similar to apple pomace extract case in this study (highest TPC for water extract; highest FRAP value for 50% aqueous ethanol), Zhang et al., (2016) reported the FRAP values of fresh Golden Delicious apple pomace extracts with methanol, ethanol, acetone, ethyl acetate, and Chloroform by using Butylated hydroxyl toluene (BHT) as standard. The highest FRAP value was obtained for ethyl acetate solvent where the highest level of phenolics was detected in the methanol extract. In another study, 70% aqueous methanol extract of dried Golden Delicious apple (1 g sample/ 20 mL solvent; 60°C; 30 min) provided approximately 1.3 ± 0.1 mg trolox equivalent/g of sample by FRAP assay and linear relationship between antioxidant capacity and total polyphenol content was obtained (Rana et al., 2021). In general, FRAP values attributed to the presence of phenolic compounds. Replacement of organic solvents with water was investigated for extraction at room temperature by Reis et al. (2012). They obtained different total phenolic content and FRAP antioxidant capacity results for dried apple pomace extracted with different solvents. Water extraction provided the highest amount of total phenolic content (1.7 mg gallic acid/ g dry apple pomace) with the highest antioxidant capacity by FRAP assay. FRAP results were 1.20, 0.30, and

0.25 mg ascorbic acid/ g dried extract for the water, methanol, and acetone extracts, respectively. Sethi et al. (2020) applied CUPRAC assay for thirteen apple cultivars except Golden Delicious apple. For this purpose, 1 g of the peel was extracted overnight with 80% methanol (sample/ solvent ratio was not reported). CUPRAC assay values were in the range of 11.99 - 46.49 mg Trolox/g peel while FRAP values were 12.62 – 48.01 mg Trolox/g peel. Their CUPRAC and FRAP result ranges were approximately in the same order of magnitude. The results obtained in our study were 0.164 - 0.310 mg GAE/ g dry apple pomace and 0.330 - 0.632 mg GAE /g dry apple pomace for FRAP and CUPRAC assays, respectively.

In the literature, different assays were used for determination of antioxidant capacities of pomaces obtained from different grape pomace varieties. As an example, freeze dried red grape (Muscat Grape) pomace was extracted 70 % aqueous ethanol (10 g pomace powder/200 mL solvent; 2 h at 70 °C with refluxing) in the study of Zhu et al., (2019). The reducing power of grape pomace extract was 1.65 ± 0.02 mmol $FeSO_4$/g powder. The result indicated that the higher reducing power of grape pomace might be related to the higher phenolic content and the stronger electrons donating abilities of the individual phenolic compounds. Furthermore, drying method, extraction techniques and origin of the grape samples were effective on total phenolic content and antioxidant capacity of the grape samples. According to our study, higher FRAP values obtained for grape pomace extraction (3.203 ± 0.117 mg GAE /mL extract water extract; 11.347 ± 0.789 mg GAE /mL extract for 50% aqueous ethanol extract; 2.588 ± 0.117 mg GAE /mL extract for ethanol extract) if compared with that of apple pomace and sour cherry pomace. In the study of Artem et al., (2021), antioxidant capacities of red grape pomace (fermented pomace of Feteasca Neagra, Cabernet Sauvignon and Mamaia) extracts were compared. The extracts were obtained by maceration (1 g of dry pomace/ 100 mL solvent; solvents: 40%, 70% aqueous ethanol solutions and ethanol ;12 days; room temperature). TPC of the extracts obtained were in the range of 0. 330 – 0.531 mg GAE/mL extract that the highest value was obtained for Cabernet Sauvignon with ethanolic extract. But, the highest FRAP values were

obtained for the extracts of red Feteasca Neagra grape pomace (0.0576 and 0.1233 mg GAE/ mL for 40% ethanol extract and 70% ethanol extract, respectively), followed by Cabernet Sauvignon (0.0421 mg GAE/mL extract and 0.0046 mg GAE/mL for 40% ethanolic extract and 70% ethanolic extract, respectively).

Although, CUPRAC method is one of the most widely used antioxidant capacity measurement methods and can be applied for both hydrophilic and hydrophobic antioxidant compounds, there are only a few study using this antioxidant capacity assay during the extraction of phenolics from sour cherry and red grape pomaces in the literature. Yammine et al., (2020) studied the characterization of polyphenols and antioxidant potential of red and white grape (Chardonnay, Cabernet Franc, Merlot and Dunkelfelder) pomace by-product extracts using subcritical water extraction (water to solid ratio was maintained at the value of 5 mL/ 1 g pomace; extraction temperature: 100 °C, 150 °C, 200 °C; pressure: $50x10^5$ Pa). For conventional extraction, 50 % aqueous ethanol (v/v) was used (liquid-to-solid ratio 5 mL/ g pomace; room temperature; 6 h). The highest CUPRAC value was obtained for Dunkelfelder variety extracted at 200 °C (493.78 ± 1.34 mg Trolox equivalent/g dry grape) while conventional extraction provided 194.34 ± 1.23 mg Trolox equivalent/g dry grape. Furthermore, in the CUPRAC assay, significant differences were found among the antioxidant capacity values of all four grape pomaces. In their study, low correlation with TPC was exhibited by FRAP and CUPRAC assays for subcritical water extraction. Manconi et al. (2016) analyzed antioxidant capacity of grape (Cannonau red grape, a Sardinian autochthonous cultivar) pomace extract by using different pressing conditions, extraction methods (maceration and homogenization) and antioxidant assays. According to the results, the highest antioxidant capacity was obtained by homogenization with the pressed grape pomaces in a 50% ethanol (1:9 solid to solvent ratio (w/v) for 24 hours, followed by sonication (23 kHz; 150 W; 16 min). FRAP and CUPRAC results were reported as 2.18 ± 0.11 mmol Fe^{2+} /g dry pomace and 5.11 ± 0.38 mmol Fe2+ /g dry pomace. Ultrasound assisted maceration and homogenization, using an ethanol/water mixture, were proposed in view of the extraction efficiency, low cost

and sustain-ability of the process. Effects of ultrasound parameters on TPC and antioxidant capacities of grape pomace (Syrah) water extracts were studied by Gonzalez-Centeno et al (2014). They found that antioxidant capacities of grape pomace water extracts increased linearly with increasing ultrasound power and processing time and similar behaviors were observed for FRAP and CUPRAC assays. TPC was found as 0.3231 mg GAE/g fresh weight with antioxidant capacities of 0.5347 and 0.4366 mg Trolox/ g fresh weight for CUPRAC and FRAP assays at the optimum extraction conditions (solid to solvent ratio of 1:5 g/mL; 40kHZ; 150 W/L ultrasound power; 25 min), respectively. There was a positive correlation between TPCs and antioxidant capacities of grape pomace extracts. This statement was in accordance with the results obtained in our study that grape pomace extracts with higher TPC provided higher antioxidant capacity for FRAP and CUPRAC assays.

In literature, the reported studies about antioxidant capacity of sour cherry pomace for FRAP and especially for CUPRAC method are limited. Antioxidant capacity values of different sour cherry cultivars were generally reported. Seven antioxidant capacity assays were compared and evaluated for the two datasets (thirteen berry genotypes and twelve sour cherry cultivars) by Rácz et al. (2015). In the case of sour cherry samples, FRAP assay was recommended to substitute all the other antioxidant capacity methods. Muchagato Maurício et al. (2020) reported that that skins (pomace without kernel) presented a higher polyphenolic content and antioxidant capacity than pomace with kernel for two different sour cherry pomace samples. Decoction in boiling water for 15 min provided a higher recovery of phenolic compounds, but, maceration with water (1 g ground sample /10 ml water) at 25 °C for 24 h was considered a more sustainable process. TPC of decoction and maceration pomace extracts were 0.173 ± 0.008 and 0.040 ± 0.004 mg GAE /g pomace, respectively. But FRAP values were determined as same (1.9 mmol Fe2+/100 g pomace) for both extraction method while DPPH•assay results were different (it was higher for decoction).

Our results showed that, the highest antioxidant capacity values were recorded for sour cherry pomace extracts with 50% aqueous ethanol which was 3.377 ± 0.236 mg GAE/ g dry pomace for FRAP assay. According to the antioxidant capacity results by FRAP assay, there is no significant difference between ethanol and water extracts. However, sour cherry pomace extracts in 50% aqueous ethanol and water solvents showed no significant difference for CUPRAC antioxidant capacity results that were 5.732 ± 0.296 mg GAE/ g dry pomace and 5.527 ± 0.314 mg GAE/ g dry pomace, respectively.

3.2.2 Total Antioxidant Capacity: DPPH$^•$ (2,2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Method

DPPH$^•$ radical is neutralized by accepting either an electron or a hydrogen atom from an antioxidant species that the radical is converted to DPPH or $DPPH_2$ according to SET and HAT mechanisms, respectively. The determination of assay reaction time is important to reach a plateau (equilibrium) for scavenging capacity (SC) in order not to underestimate the results (Mishra et al.,2012). The plateau reaction time was determined as 60 min for GA standard in the concentration range of 0.001- 0.05 mg/mL. In the literature, the most frequently reported reaction times were in the range of 30 - 60 min to reach a plateau. DPPH$^•$ assay has been applied to quantify antioxidant capacity in foods and plant extracts using different antioxidant standards such as ascorbic acid, butylated hydroxyl toluene (BHT), α-tocopherol, butylated hydroxyl anisole (BHA), gallic acid and Trolox. Fadda et al. (2014) reported different reaction kinetics for different standards and plant extracts. Moreover, while keeping the same antioxidant/DPPH$^•$ ratio, the time necessary to reach equilibrium was dependent on the initial DPPH$^•$ concentration, showing that longer time intervals were required when using lower DPPH$^•$ concentrations.

This assay often presented inconsistencies in the literature due to the use of different standards and also the mistake done in the determination of the percentage of scavenged DPPH$^•$. DPPH$^•$ SC and antioxidant standard concentration

relationship was not linear for GA standard in methanol (Figure 3.1). The change seemed as linear up to 0.01 mg GA/mL (0.06 mg GA/mg of $DPPH^{\bullet}$), but after this GA concentration, the change was not linear. Therefore, great attention is required when considering the dilution factors, the incorrect results can be obtained if a linear calibration curve is used for the estimation of SC and also each sample would need its own calibration curve. Increasing GA concentrations up to 0.05 mg/mL, resulted in the maximum SC of 72.04 ± 2.57 %. Lu et al. (2014) determined 91.50 ± 0.01 % $DPPH^{\bullet}$ equilibrium inhibition after 0.008 mg GA/mL that the $DPPH^{\bullet}$ assay was applied for 20 minutes at 37˚C by preparing GA standards (0.001-0.01 mg/mL) in chloroform. Linearity of GA in ethanol was obtained below 0.01 mg GA/mL and approximately maximum 80 % SC was obtained in the study of Carmona-Jiménez et al. (2014) that was in accordance with the results obtained in our study. De Menezes et al., (2021) suggested the molar or mass ratio of antioxidant/$DPPH^{\bullet}$ would be the correct choice for comparison the results from different sources. Figure 3.1 presents the change of $DPPH^{\bullet}$ SC with respect to GA concentration. Due to this nonlinearity, $DPPH^{\bullet}$ radical scavenging effect can be quantified in terms of EC_{50} value that is the antioxidant concentration required to obtain a 50% SC. A lower value of EC_{50} indicates higher antioxidant effectiveness. EC_{50} values were initially used for drugs and calculated from log concentration–response curve (logarithmic model). Once the EC_{50} values fall in a linear range, it may be easily calculated from by using linear regression. If it is in non-linear range, the estimation of the EC_{50} may be quite difficult. This lack of linearity means that it is necessary to study the behavior of each sample and to obtain sample standard curve. For this purpose, several aliquots containing different sample concentrations must be tested and then the EC_{50} value is calculated from the mathematical model of this nonlinear calibration curve.

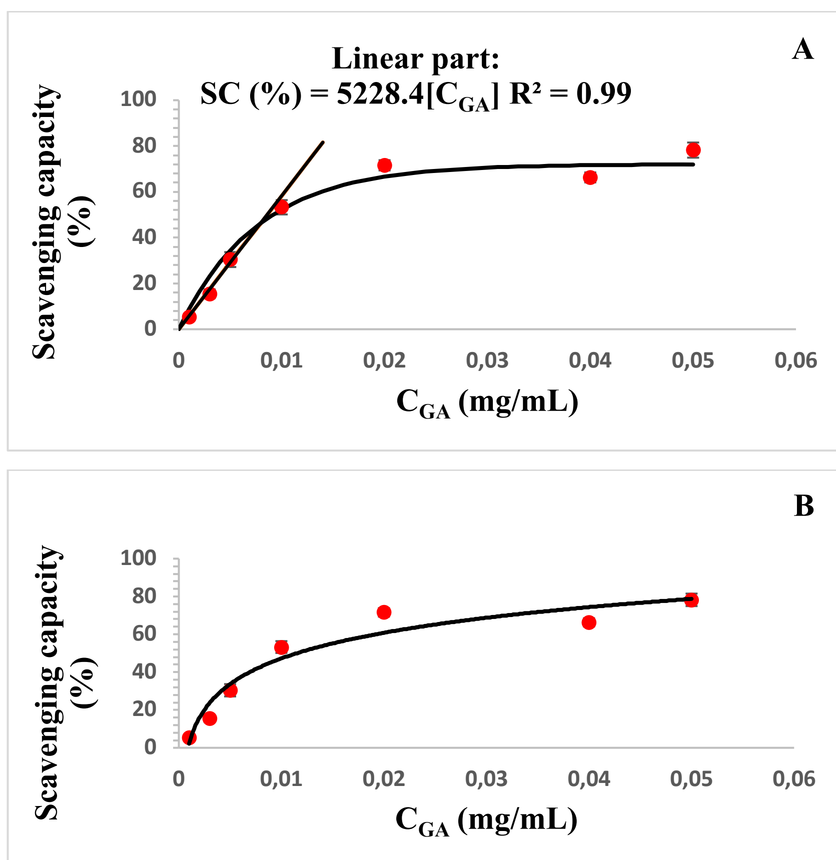


Figure 3.1 Presentation of the scavenging capacity for GA standard in methanol

A: exponential distribution model (equation 3.2) α = 129.12 mL/ mg GA (R^2=0.98)

B: Logarithmic model (equation 3.3) SC = 19.58 ln(C_{GA})+ 137.41 (R^2 = 0.94)

Some studies showed the lack of correlation between the DPPH$^\bullet$ SC and the concentration of the antioxidants for the estimation of EC_{50} value or in order to use the linear change range in the calibration curve, determining the EC_{20} values instead of EC_{50}, was proposed (Carmona-Jiménez et al., 2014; Goujot et al., 2019; Romanet et al., 2021). Figures 3.2, 3.3 and 3.4 shows SC values of apple, sour cherry and grape pomace extracts at different TPC values. Table 3.4 shows EC_{50} and EC_{20} values of the sample extracts. These values were calculated from the logarithmic model.

Sethi et al (2020) analyzed thirteen apple cultivars for antioxidant capacity of methanol extracts of peel and cortex fractions (1g of the peel or cortex of each was

extracted overnight with 80% methanol at room temperature). Apple cortex showed very low DPPH$^{\bullet}$ scavenging capacity (average 2.35%) in comparison to peel fractions (average 274.82 % that is higher than 100%). The maximum SC was obtained 89.34 ± 0.11 % for apple pomace 50 % aqueous ethanol extract in our study. Sethi et al (2020) also reported that the antioxidant capacity was best expressed by FRAP assay. According to the study of Rana et al., (2021), the antioxidant capacity of 70% aqueous methanol Golden Delicious pomace extract was determined as 2.5 ± 0.10 mg trolox equivalent/g dry powder. In our study, the extract obtained with 50 % ethanol provided 0.821 ± 0.001 mg GAE/g pomace that it was out of the GA calibration curve. Zhang (2016) reported that DPPH$^{\bullet}$ assay results were varied according to the extraction medium of Golden Delicious apple pomace (100 g flesh apple pomace /500 ml solvent at 37 °C for 40 min). The results were 2.13 ± 0.13, 2.11 ± 0.10, 1.19 ± 0.11, 3.05 ± 0.14 and 1.09 ± 0.08 mg BHT equivalents / g of dry extract powder for methanol, ethanol, acetone, ethyl acetate and chloroform extracts, respectively. Similarly, in our study, different results were obtained for DPPH$^{\bullet}$ assay according to the solvent type used. In the study of Wang et al. (2015), EC_{50} value of 70 % methanol peel extract of Golden Delicious apple extract was 61.7 ± 5.5 mg trolox equivalent /ml that was much higher than that calculated for positive control, trolox (0.235 mg/mL). Such a higher difference was not observed in our study (EC_{50} = 0.021 mg GAE/mL for apple pomace 50% ethanol extract, EC_{50} = 0.012 mg GA/mL for GA standard in methanol). Therefore, the assay reactional composition and reaction time affects the results reported.

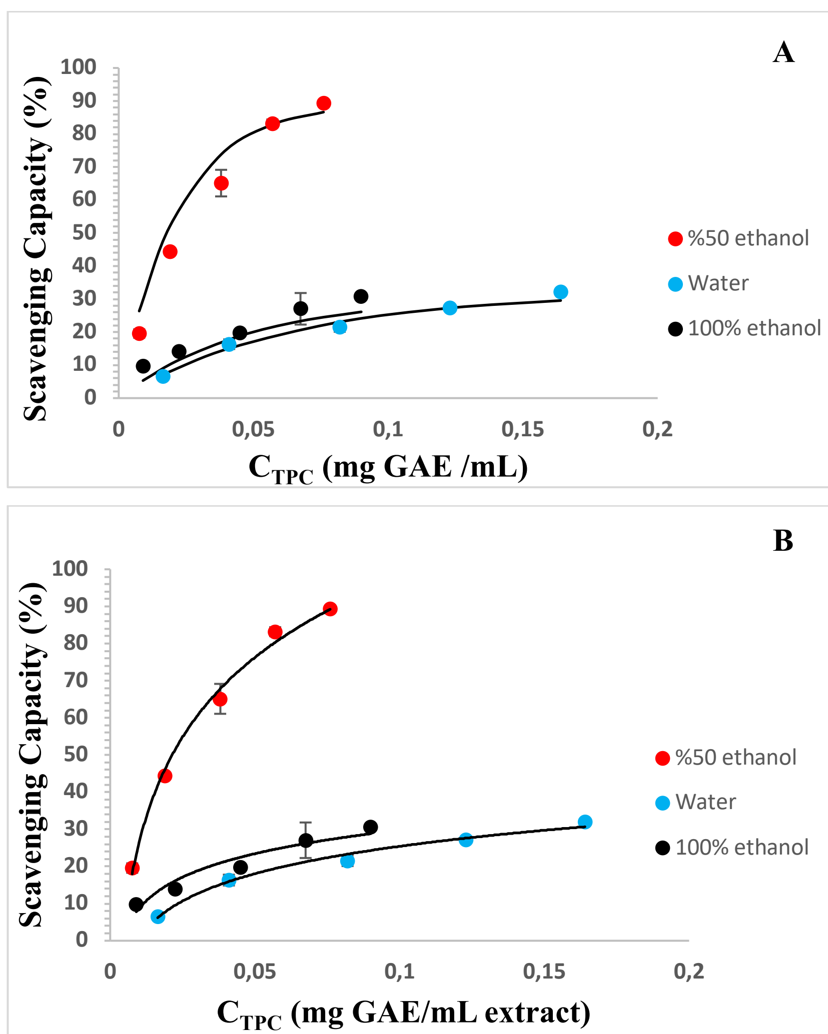


Figure 3.2 Scavenging capacity presentation of apple pomace extracts

A: exponential distribution function (equation 3.2)

Water extract: $\alpha = 15.45$ mL/ mg GAE (R^2=0.999)

50 % aqueous ethanol extract: $\alpha = 46.02$ mL/ mg GAE (R^2=0.983)

Ethanol extract: $\alpha = 21.10$ mL/ mg GAE (R^2=0.972)

B: Logarithmic model (equation 3.3):

Water extract: $SC = 10.637 \ln(C_{TPC}) + 49.913$ ($R^2 = 0.986$)

50 % aqueous ethanol extract: $SC = 30.947 \ln(C_{TPC}) + 168.98$ ($R^2 = 0.994$)

Ethanol extract: $SC = 9.1249 \ln(C_{TPC}) + 50.776$ ($R^2 = 0.935$)

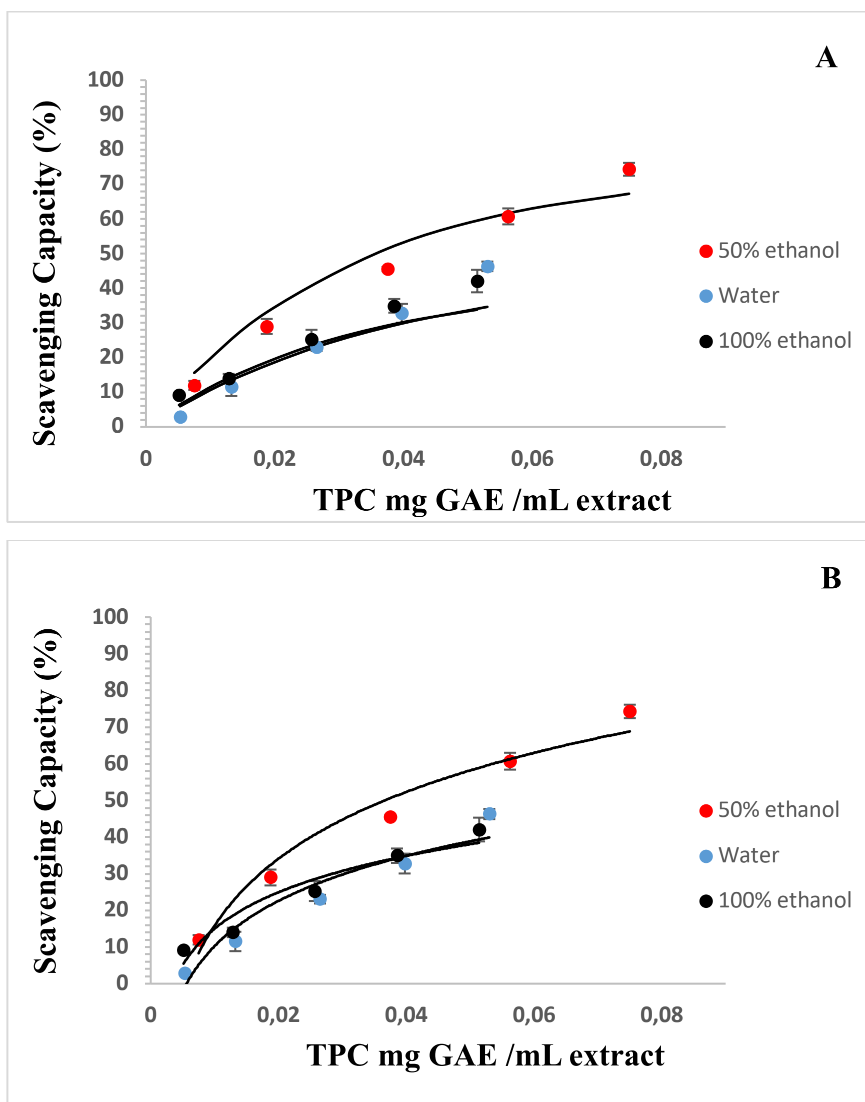


Figure 3.3 Scavenging capacity presentation of sour cherry pomace extracts

A: exponential distribution model (equation 3.2)

Water extract: α = 25.988 mL/ mg GAE (R^2=0.990)

50 % aqueous ethanol extract: α = 31.409 mL/ mg GAE (R^2= 0.979)

Ethanol extract: α = 31.695 mL/ mg GAE (R^2=0.995)

B: Logarithmic model (equation 3.3):

Water extract: $SC = 17.773 \ln(C_{TPC}) + 92.105$ (R^2 =0.920)

50 % aqueous ethanol extract: $SC = 26.298 \ln(C_{TPC}) + 136.97$ (R^2 = 0.967)

Ethanol extract: $SC = 14.325 \ln(C_{TPC}) + 80.983$ (R^2 = 0.923)

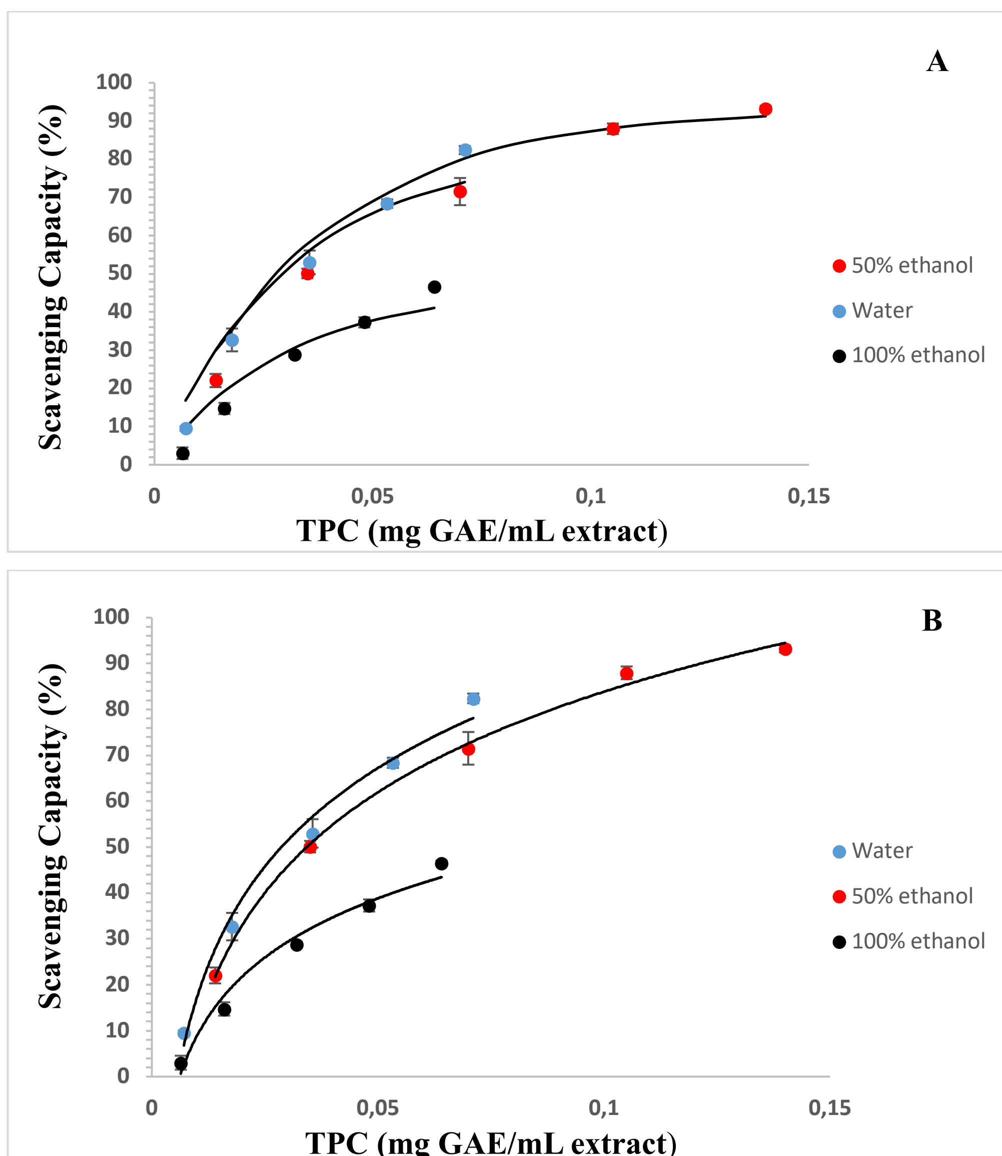


Figure 3.4 Scavenging capacity presentation of grape pomace extracts

A: Exponential distribution model (equation 3.2)

Water extract: α = 32.143 mL/ mg GAE (R^2=0.996)

50 % aqueous ethanol extract: α = 27.657 mL/ mg GAE (R^2= 0.981)

Ethanol extract: α = 33.404 mL/ mg GAE (R^2=0.983)

B: Logarithmic model (equation 3.3):

Water extract: SC = 30.977 ln(C_{TPC}) + 160.02 (R^2 =0.986)

50 % aqueous ethanol extract: SC = 31.609 ln(C_{TPC}) + 156.63 (R^2 = 0.997)

Ethanol extract: SC = 18.588 ln(C_{TPC}) + 94.514 (R^2 = 0.977)

Table 3.4 Antioxidant capacity results of fruit pomace extracts according to DPPH method

Solvent	DPPH• antioxidant capacity SC_{max} (%)	 EC_{50} C_{TPC} (mg GAE) / mL extract	 EC_{20} C_{TPC} (mg GAE) / mL extract)
		Apple pomace	
water	32.10 ± 0.28	SC_{max} < 50 %	0.0612 ± 0.0011[a*]
50 % aqueous ethanol	89.34 ± 0.11	0.021 ± 0.000	0.0067 ± 0.0000[c]
ethanol	30.72 ± 0.66	SC_{max} < 50 %	0.0419 ± 0.0071[b]
		Sour cherry pomace	
water	46.30 ± 1.39	SC_{max} < 50 %	0.0172 ± 0.0001[a]
50 % aqueous ethanol	74.29 ± 1.84	0.039 ± 0.002	0.0125 ± 0.0007[c]
ethanol	42.10 ± 3.24	SC_{max} < 50 %	0.0143 ± 0.0002[b]
		Grape pomace	
Water	82.40 ± 0.79	0.0245 ± 0.0037[b]	0.0115 ± 0.0016[a]
50 % aqueous ethanol	93.20 ± 1.04	0.0347 ± 0.0005[a]	0.0162 ± 0.0029[a]
ethanol	46.54 ± 051	SC_{max} < 50 %	0.0157 ± 0.0109[a]

* different letters within the same column shows significance difference ($p \leq 0.05$)

For sour cherry pomace, 50% aqueous ethanol extract provided the highest antioxidant capacity (0.0125 mg GAE/mL) according to DPPH• assay. Ciccoritti et al. (2018) studied sour cherry pomace (two local varieties, Italy) extract antioxidant capacity. During their study, the extraction was performed with 80% aqueous methanol (1 g dry pomace/15 mL solvent) at room temperature for 30 min and then followed by 30 min of UAE. The extraction procedure was repeated twice using 15 mL of fresh solvent. EC_{50} values were determined as 0.49 ± 0.08 and 1.02 ± 0.05 mg dry sample for different varieties and significant negative correlations were found between EC_{50} and TPC values. This value was approximately 0.26 mg dry pomace for 50% aqueous ethanol extract and showed better antioxidant capacity obtained in our study. Simsek et al (2012) reported DPPH• EC_{50} valuee of freeze dried sour cheery pomace extracts obtained by conventional extraction (30mL /g

solvent to solid ratio; 700 W heating power; 6 h). Their results are in accordance with the result obtained from our study that 50 % aqueous ethanol solvent provided the highest antioxidant capacity in extracts. They reported EC_{50} values were 0.040, 0.050 and 0.056 g sample/mg DPPH for ethanol-water, ethanol, and water solvents, respectively. This value was approximately 0.104 g pomace/ mg $DPPH^{\bullet}$ for 50% aqueous ethanol sour cherry pomace extract in our study. Okur et al (2019) reported 85.77 ± 0.36 % $DPPH^{\bullet}$ inhibition (TPC: 2.40 ± 0.0289 mg GAE/ g fresh weight) for UAE (24 kHz; 100 % amplitude (100%); 80% aqueous methanol; 1g pomace / 10 mL solvent; 15 min) of sour cherry pomace extract. In our study, maximum SC measured not over 80 % for 50% aqueous ethanol extract containing TPC of 7.492 ± 0.462 mg GAE/ g dry pomace). SC comparison of different studies cannot be done, therefore the calculation of EC values may be more suitable. But, as mentioned before, Fadda et al. (2014) reported that while keeping the same antioxidant/$DPPH^{\bullet}$ ratio, the time necessary to reach a constant value was dependent on the initial $DPPH^{\bullet}$ concentration, showing that longer time intervals were required when using lower $DPPH^{\bullet}$ concentrations. Therefore, the reaction time to reach to plateau must be carefully determined.

Other pomace studied was grape pomace extract. Yeler & Nas (2020) studied the optimization of phenolic extraction from dried red grape (öküz gözü) pomace. Ethanol/0.1% citric acid solutions (70:30 and 50:50, v/v) were used as solvents during extraction (1g sample /12mL solvent at 30, 40 and 50 °C for 30, 90, 150 and 180 min). EC_{50} values of dried grape pomace extracts ranged from 1.99 to 3.65 mg dry sample /mL, For our case, EC_{50} values were 0.0245 and 0.0347 mg GAE/mL for water and 50% aqueous ethanol extracts, respectively. These numbers correspond 1.723 and 1.238 mg dry pomace/mL for water and 50% aqueous ethanol extracts. Iora et al (2015) reported the antioxidant capacity of three different grape varieties (Merlot, Tanat and Cabernet Sauvignon) pomaces for extraction with 40% ethanol (solute/solvent ratio of 1:20 (w/v) at room temperature for 24 h). DPPH assay ranged from 5.05 to 6.54 mg dry pomace/ mL for ethanolic extracts of grape pomace.

Romanini et al. (2021) studied the effects of extraction methods on phenolic content and antioxidant capacity of a hybrid cultivar (BRS Violet) grape pomace. Ultrasound assisted extraction (750 Watts and 20 kHz; 1g sample /200mL water; 55 °C; 6 min) and conventional extraction with water, were used in their study. According to $DPPH^{\bullet}$ antioxidant capacity, application of ultrasound proved the extraction of antioxidants (0.23 mmole Trolox /g grape peel for UAE and 0.12 mmole Trolox /g grape peel for conventional extraction). They reported water as a suitable for extraction. The variety used in our study was Kalecik karası grape. According this assay, for the dried and defatted seed and dried skin powder extracts, antioxidant scavenging capacity increased in the following order: Narince skin extract < Cabernet Sauvignon skin extract <Kalecik karası skin extract (37.42±1.87%) <Narince seed extract < Cabernet Sauvignon seed extract< Kalecik karası seed extract (92.90±4.64 %) in the study of Baydar et al., (2011). Soxhlet extraction (1 g sample/2 ml solvent) was done with acetone: water: acetic acid solvent (90:9.5:0.5, v/v) at 60°C for 8 h.

The highest TPC for grape pomace extracts was obtained with 50% aqueous ethanol (2.803 ± 0.205 mg GAE/mL) if compared with that of water (1.423 ± 0.126 mg GAE/mL) for this study. Higher SC_{max} value was also obtained with 50% aqueous ethanol extract (93.20 %) if compared with that of water (82.40%). However, EC_{20} value of water extraction (0.0115 ± 0.0016 mg GAE/mL) was lower than that of 50% ethanol extraction (0.0162 ± 0.0029 mg GAE/mL). In other words, grape pomace extract with water showed slightly higher antioxidant capacity. This unexpected result was not in accordance with the results reported by authors who indicated a positive trend between antioxidant capacity and TPC. (Rockenbach et al., 2011; Xu et al., 2010). However, others researchers expressed that the antioxidant capacity was dependent on the phenolic profile (Karasu et al., 2016; Lutz et al., 2011; Baiano & Terracone, 2011). The absorption of anthocyanins is maximum at the same wavelength range of the assay and this cause interference with the results reported (Shaidi & Zhong, 2015). Another explanation can be that the presence of water in reactional medium may cause turbidity by

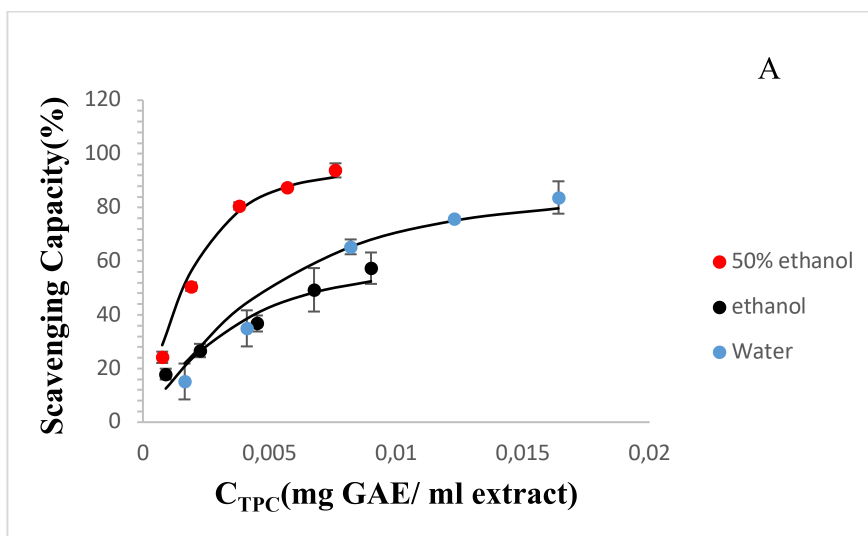
interacting with methanolic $DPPH^{\bullet}$ as reported by Dawidowicz et al. (2012). They detected increasing antioxidant capacity of examined compound estimated by $DPPH^{\bullet}$ method with increasing water content in reactional medium.

For this study, exponential distribution and logarithmic models were used. This models provided higher R^2 values. This results were accordance in the study by Gonzales-Centeno et al. (2015). They reported that modified Weibull model was suitable to accurately predict the extraction kinetics of phenolics and antioxidant capacity from grape pomace within the range of temperatures 20–50 °C.

3.2.3 $ABTS^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Radical Scavenging Assay

Similar to $DPPH^{\bullet}$ assay calculation procedure, $ABTS^{\bullet+}$assay results were presented in Figure 3.6, 3.7. Table 3.5 presents antioxidant capacity results (EC_{50} and EC_{20}) of fruit pomace extracts according to $ABTS^{\bullet+}$ method. 50% aqueous ethanol provided lowest EC_{20} values that were different if compared with that of $DPPH^{\bullet}$assay.

$ABTS^{\bullet+}$ in the aqueous buffer solution reaches equilibrium value within 30 min. The reaction was slow when $ABTS^{\bullet+}$ reacted with antioxidants in alcohol. Gallic acid reached a stable end point immediately in the $DPPH^{\bullet}$ assay but required more than 60 min to approach the end point in the FRAP and $ABTS^{\bullet+}$ assays. Fruit samples of raspberry, blackberry, grape and strawberry extracts required long reaction times to approach to the end point. In the $ABTS^{\bullet+}$ assay, the antioxidant capacity (trolox equivalent) of quercetin and gallic acid was approximately three times higher than chlorogenic acid or caffeic acid and six times higher than ascorbic acid or trolox after a 120 min reaction. These differences in antioxidant capacity among the standards were not as great in the FRAP and $DPPH^{\bullet}$ assays (Özgen et al., 2006).



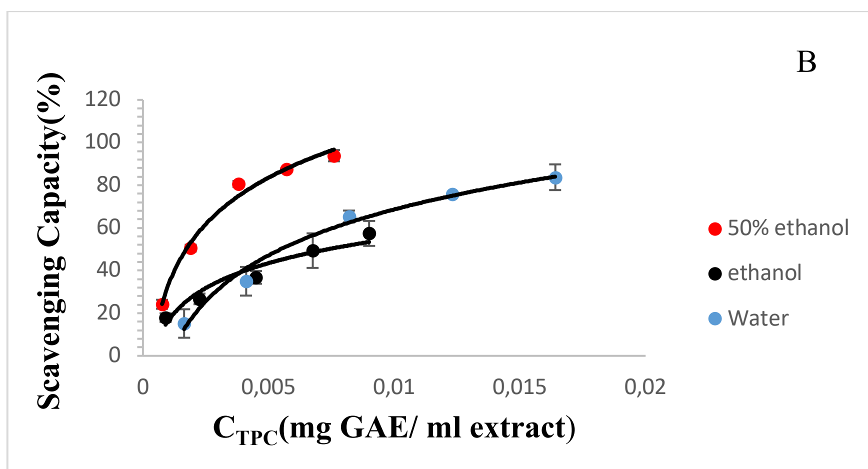


Figure 3.5 Scavenging capacity presentation of apple pomace extracts

A: Exponential distribution model (equation 3.4):

Water extract: α = 184.72 mL/ mg GAE (R^2=0.992)

50 % aqueous ethanol extract: α = 480.55 mL/ mg GAE (R^2= 0.995)

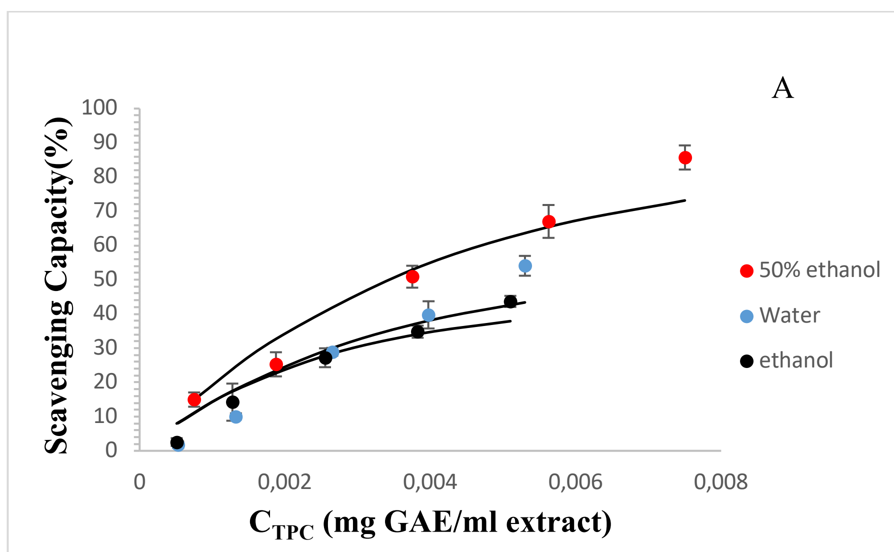
Ethanol extract: α = 273.45 mL/ mg GAE (R^2=0.987)

B: Logarithmic model (equation 3.3)

Water extract: SC = 31.052 ln(C_{TPC}) + 211.65 (R^2 =0.985)

50 % aqueous ethanol extract: SC = 31.445 ln(C_{TPC}) + 250.15 (R^2 = 0.986)

Ethanol extract: SC = 16.852 ln(C_{TPC}) + 132.75 (R^2 = 0.939)



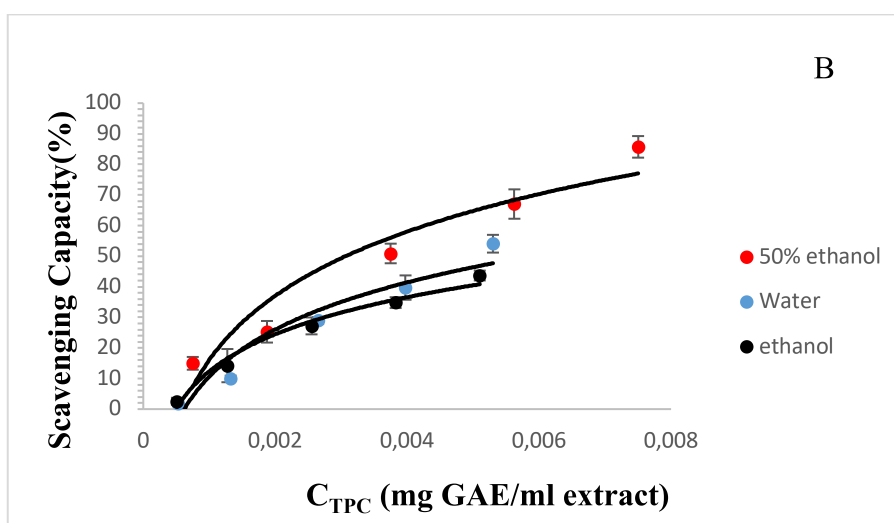


Figure 3.6 Scavenging capacity presentation of sour cherry pomace extracts

A: Exponential distribution model (equation 3.6):

Water extract: α = 305.65 mL/ mg GAE (R^2=0.971)

50 % aqueous ethanol extract: α = 255.97 mL/ mg GAE (R^2= 0.992)

Ethanol extract: α = 393.46 mL/ mg GAE (R^2=0.989)

B: Logarithmic model (equation 3.5)

Water extract: SC = 22.221 ln(C_{TPC}) + 164.11 (R^2 =0.929)

50 % aqueous ethanol extract: SC = 30.271 ln(C_{TPC}) + 225.15 (R^2 = 0.924)

Ethanol extract: SC = 17.483 ln(C_{TPC}) + 133.1 (R^2 = 0.939)

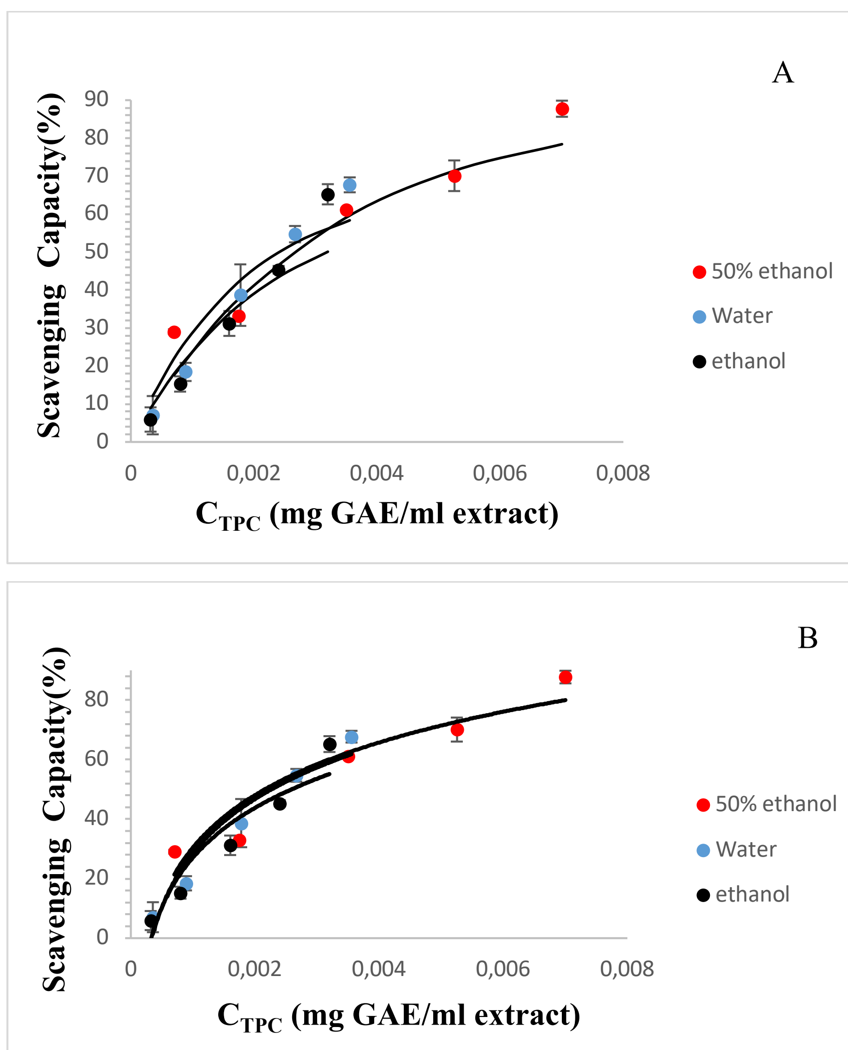


Figure 3.7 Scavenging capacity presentation of grape pomace extracts

A: Exponential distribution model (equation 3.8):

Water extract: α = 556.81 mL/ mg GAE (R^2=0.976)

50 % aqueous ethanol extract: α =319.90 mL/ mg GAE (R^2= 0.989)

Ethanol extract: α = 456.58 mL/ mg GAE (R^2=0.986)

B: Logarithmic model (equation 3.9)

Water extract: $SC = 26.249 \ln(C_{TPC}) + 209.86$ (R^2 =0.944)

50 % aqueous ethanol extract: $SC = 25.471 \ln(C_{TPC}) + 206.39$ (R^2 = 0.894)

Ethanol extract: $SC = 24.150 \ln(C_{TPC}) + 193.88$ (R^2 = 0.892)

The calibration curve of $ABTS^{\bullet+}$assay was reported as linear in the range of 0.27 x 10^{-3} - 1.36 x 10^{-3} mg GA/mL (Abramovic et al.,, 2018) that in accordance with result obtained in this study (Figure 2.4). Rana et al. (2021) extracted dry pomaces of five apple varieties: Royal Delicious, Golden Delicious, Red Chief, Red Delicious and Red Gold, with 70% aqueous methanol (1 g powder/20 ml at 60°C for 30 min) and analyzed the extracts for their antioxidant capacity. The trolox equivalent antioxidant capacity (mg/g dry powder) value of five varieties ranged from 2.29 ± 0.09 to 3.35 ± 0.10 for $DPPH^{\bullet+}$ assay, 3.68 ± 0.03 to 4.67 ± 0.03 for $ABTS^{\bullet+}$ assay and 0.95 ± 0.11 to 2.71 ± 0.10 for FRAP assay that he antioxidant capacity was low for Golden Delicious apple pomace but Graziani et al. (2021) reported antioxidant capacity of Golden Delicious apple peel as 24.72 ± 0.77 and 58.90 ± 4.40 mmole trolox/kg DW for $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays, respectively. The results cannot be compared with our results obtained with 50% aqueous ethanol due to the difference in calculation procedure. Since they used linear calibration curves. But, according to the our results, Golden delicious apple pomace extract provided good antioxidant capacity if compared with that of the sour cherry pomace extracts. DPPH assay is also sensitive to the presence of water and pH. The increase in the concentration of hydrogen ions cause a decrease in the antioxidant capacity due to the decrease of the antioxidant/$DPPH^{\bullet}$ reaction rate (Dawidowicz & Olszowy, 2012; Pekal & Pyrzyńska, 2015). For plant based extracts, the precipitation can be observed due to mixing of the sample with the alcoholic medium of the $DPPH^{\bullet}$. None of the aliquots used in our study showed precipitation problem. The $ABTS^{\bullet+}$ assay can be used at different pH levels, unlike DPPH which is sensitive to acidic pH. Additionally, $ABTS^{\bullet+}$ is soluble in aqueous and organic solvents. In the study of Wang et al. (2015), $DPPH^{\bullet+}$ assay EC_{50} value of 70 % methanol peel extract of Golden Delicious apple was 61.7 ± 5.5 mg trolox equivalent /mL extract that was much higher than that calculated for positive control, Trolox (0.235 mg/mL). The antioxidant capacities in extracts was also determined as Trolox equivalents by using the $ABTS^{\bullet+}$ (45.2 ± 0.9 µmol trolox

equivalent /g fresh pomace) and EC_{50} value estimation was not done. High correlation was obtained between TPC and $ABTS^{\bullet+}$ antioxidant capacity.

Table 3.5 Antioxidant capacity results of fruit pomace extracts according to $ABTS^{\bullet+}$ method

		$ABTS^{\bullet+}$ assay results	
Solvent	**SC_{max} (%)**	**EC_{50} mg TPC (GAE)/ml extract**	**EC_{20} mg TPC (GAE) /mL extract**
		Apple pomace	
Water	84.74 ± 6.03	$4.21x10^{-3} \pm 0.21\ x10^{-3b*}$	$1.67x10^{-3} \pm 8.52\ x10^{-5a}$
50 % aqueous ethanol	93.85 ± 2.63	$1.71x10^{-3} \pm 0.12\ x10^{-3c}$	$0.66x10^{-3} \pm 5.21\ x10^{-5\ c}$
Ethanol	57.40 ± 5.85	$7.63x10^{-3} \pm 0.28\ x10^{-3a}$	$1.28x10^{-3} \pm 4.29\ x10^{-5\ b}$
		Sour cherry pomace	
Water	54.06 ± 2.89	$5.93\ x10^{-3} \pm 9.72\ x10^{-5a}$	$1.55\ x10^{-3} \pm 3.94\ x10^{-5a}$
50 % aqueous ethanol	85.71 ± 3.53	$3.31\ x10^{-3} \pm 6.81\ x10^{-5b}$	$1.13\ x10^{-3} \pm 4.39\ x10^{-6b}$
Ethanol	43.66 ± 1.61	SC_{max} <%50	$1.54\ x10^{-3} \pm 2.65\ x10^{-5a}$
		Grape pomace	
Water	67.71 ± 1.96	$2.21\ x10^{-3} \pm 11.5\ x10^{-5b}$	$9.91\ x10^{-4} \pm 3.99\ x10^{-4a}$
50 % aqueous ethanol	87.75 ± 2.12	$2.06\ x10^{-3} \pm 7.86\ x10^{-5b}$	$6.71\ x10^{-4} \pm 9.31\ x10^{-6a}$
Ethanol	65.22 ± 2.66	$2.56\ x10^{-3} \pm 3.34\ x10^{-5a}$	$7.46\ x10^{-4} \pm 8.02\ x10^{-7a}$

* different letters within the same column shows significance difference ($p \leq 0.05$)

Antioxidant capacity of grape pomace by $ABTS^{\bullet+}$ and the correlation with total phenolic content were studied by several authors. Xu et al (2010) reported antioxidant capacity values of different grape cultivars grown in China. The highest antioxidant capacity by $ABTS^{\bullet+}$ assay (488.86 ± 12.14 µmol trolox equivalent /g dry weight) was found for *Cabernet Sauvignon* pomace extract with methanol/water/acetic acid solvent (70:29:1, v/v/v). They reported that antioxidant capacity values can only be compared when the measurements are made with the

same method and the effects of solvent should be tested first. As an example, acidic methanolic solvent was could also contribute

to the reduction of the radicals in both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays, causing an overestimation of the antioxidant capacity of phenolic and, to eliminate this interference, the extracts must be diluted. Another important factor reported was the nonlinearity of calibration curves. In our study, grape pomace extract with 50% ethanol solvent had the highest TPC value among water and ethanol solvents but there was no significant difference between EC_{20} values of grape pomace extracts with 50% ethanol, water and ethanol solvent (Table 3.5).

Sridhar and Charles (2019) studied the grape skin, seed, and, flesh extracts. Extractions were performed with acetone: water (4:1 v/v), water, and 75% ethanol: water (4:1 v/v) by UAE for 60 min at room temperature by analyzing the prediction of EC_{50} values of Kyoho grape skin, seed, and flesh extracts obtained by $DPPH^{\bullet+}$ and $ABTS^{\bullet+}$ assays. Their most obvious finding was that $DPPH^{\bullet}$ and $ABTS^{\bullet}$ assays were not comparable for extracts. Non-linear regression models can be recommended for the estimation of EC_{50} values. The $DPPH^{\bullet}$ results of grape extracts exhibited a different shape of the curve (non-linear), while $ABTS^{\bullet+}$ method showed a dose-response curve. According to the study of Da Rocha and Norena (2020), the grape pomace mix (70 % Isabel, 15 % Bordo, 10 % Carmem and 5 % Niagara) were extracted with water acidified with 2 % (w/v) citric acid (1:3, w/w). Microwave assisted extraction (1000 W for 10 min) provided antioxidant capacities as 23.84 ± 0.57 and 33.27 ± 2.00 μmol trolox equivalent / g dry pomace, respectively for $ABTS^{\bullet+}$ and $DPPH^{\bullet}$assays, respectively. These values for UAE (450 W, 15 min) were approximately 20 and 25 μmol trolox equivalent / g dry pomace for $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays, respectively. Their results showed that when acidified water solution is used as a solvent, the phenolic content and antioxidant capacity were lower when compared with extraction using methanol acidified solution. According to Table 3.5, extraction with water and ethanol provided the same antioxidant capacity according to $ABTS^{\bullet+}$ EC_{20} value.

Several studies that were reported by several authors in the literature indicated that TPC was correlated with $ABTS^{\bullet+}$ antioxidant capacity for sour cherry pomace extract. Khoo et. al. (2011) reported antioxidant capacity of 34 sour cherry cultivars (2 g cherry /1 mL water extract). They found antioxidant capacities in a range of 9-63 µmol trolox equivalent /g fresh cherry. They claimed that antioxidant capacity was highly correlated to TPC. Similarly, Wojdylo et. al. (2014) confirmed the correlation between TPC and antioxidant activity of sour cherries by $ABTS^{\bullet}$ assay (3.72-18.40 mmol trolox equivalent/ 100g dry weight). Ciccoritti et al. (2018) studied sour cherry pomace (two local varieties, Italy) extract antioxidant capacity. During their study, the extraction was performed with 80% aqueous methanol (1 g dry pomace/15 mL solvent) at room temperature for 30 min and then followed by 30 min of UAE. The extraction procedure was repeated twice using 15 mL of fresh solvent. For $DPPH^{\bullet}$ EC50 values were determined as 0.49 ± 0.08 and 1.02 ± 0.05 mg dry sample for two different varieties and significant negative correlations were found between $DPPH^{\bullet}$ EC_{50} and TPC values. For $ABTS^{\bullet+}$assay, EC_{50} values were 0.07 ± 0.01 and 0.21 ±0.01 mg dry sample for two different varieties. The authors reported a remarkable correlation between TPC and $ABTS^{\bullet+}$ values. Demirdöven et al., (2015) applied $ABTS^{\bullet+}$ test for the analysis of sour cherry pomace extracts obtained by conventional extraction (optimum conditions: 42.39% aqueous ethanol: 1/15 w/v ratio at 40°C for 75 min) and ultrasonic extraction (optimum conventional extraction parameters at 37 kHz). TPC was found in conventional extraction sample as 16.320 ± 5.24 mg GAE /L, while it was 15.470 ± 7.43 mg GAE /L in UAE. Antioxidant capacity of UAE samples was found as 41.27 ± 0.24 mm trolox /mL and it was 36.25 ± 0.32 mm trolox/mL for conventional extraction. High antioxidant activity values of UAE samples were explained by the extraction of other antioxidants such as vitamins. For our study, the extraction with 50% aqueous ethanol also provided the lowest EC_{50} and EC_{20} values.

3.3 Pearson correlation coefficients

Table 3.6 shows Pearson correlation analysis of TPC and antioxidant capacity methods applied for all pomaces and solvents.

Table 3.6 Pearson correlation analysis of TPC and antioxidant capacity methods

	TPC	**FRAP**	**CUPRAC**	**ABTS$^{\bullet+}$ SC$_{max}$**	**ABTS$^{\bullet+}$ EC$_{20}$**
FRAP	0.950*				
CUPRAC	0.954*	0.885*			
ABTS$^{\bullet+}$ SC$_{max}$	0.200	0.276	0.071		
DPPH$^{\bullet}$ SC$_{max}$	0.566	0.594	0.536	0.629	
DPPH$^{\bullet}$ EC$_{20}$	-0.361	-0.337	-0.494		0.581
ABTS$^{\bullet+}$ EC$_{20}$	-0.549	-0.480	-0.458		
ABTS$^{\bullet+}$ EC$_{50}$	-0.511	-0.426	-0.482		0.718**

*: significant at $p < 0.01$; **: significant at $p < 0.05$

Metal ion reducing capacity assays, FRAP and CUPRAC values were significantly ($p < 0.01$) correlated with TPC. Significant correlation between CUPRAC and FRAP assays was also obtained ($r = 0.885$, $p < 0.01$), therefore one of them can be selected for antioxidant capacity estimation. Seven antioxidant capacity assays were compared and evaluated for the two datasets (thirteen berry genotypes and twelve sour cherry cultivars) by Rácz et al. (2015). In the case of sour cherry samples, FRAP assay was recommended to substitute all the other antioxidant capacity methods in their study. The principle of CUPRAC method is similar to that of the FRAP method that ferric ion is replaced by cupric ion. Although, CUPRAC method can be applied for both hydrophilic and hydrophobic antioxidant capacity estimation. Özyürek et al., (2011) observed relatively independent solvent effects in alcohol–water mixtures of varying composition for a wide standard concentration range for CUPRAC assay.

The correlations between DPPH$^•$ EC_{20} values and TPC was obtained as a low correlation (r = -0.361). Low correlations were also obtained between DPPH$^•$ EC_{20} and metal ion reducing capacity assays. Similar low correlation was obtained for ABTS$^{•+}$ EC_{20} and EC_{50} values. Yemis, Bakkalbasi, and Artik (2008) investigated the antioxidant capacities of different grape variety seed extracts with 70% aqueous acetone and reported weak or no correlation between DPPH$^•$ and ABTS$^{•+}$ assays in terms of trolox equivalent values. But the significant correlation between TPC and DPPH$^•$ trolox equivalent was obtained as 0.7974 ($p< 0.001$) in their study that was not in accordance with the results obtained in our study. Low non-significant correlation (0.4860) was obtained for TPC-ABTS$^{•+}$ trolox equivalent values in their study similar to obtained in this study. The most obvious finding from their study was trolox equivalent values that DPPH$^•$ and other antioxidant capacity assays not comparable for extracts.

Pearson analysis was also applied to the set of data measured for each pomace extracts (Table 3.7). Generally, higher correlations between TPC and antioxidant assays were obtained for grape and for sour cherry pomace extracts. For apple pomace extracts, TPC- FRAP and TPC-CUPRAC and FRAP-CUPRAC assays' correlations were low. Meanwhile the TPC is in a negative correlation with the antioxidant capacity measured by FRAP method, since higher TPC was obtained by extraction with water, while higher antioxidant capacity was obtained by extraction with 50 % aqueous ethanol. Generally, for sour cherry and grape pomace extracts, FRAP assay provided a high correlation with the other assays. CUPRAC and ABTS$^{•+}$ methods, having monopositive charged chromophores, can simultaneously measure hydrophilic and lipophilic antioxidants, whereas FRAP method having either hydrophilic or multicharged chromophores require the enhancement for the solubilization of lipophilics phenolics. A mixed-mode (HAT/SET) free radical scavenging assays such as the DPPH$^•$ assay is very sensitive to reactional medium composition.

Table 3.7 Pearson correlation analysis of TPC and antioxidant capacity methods for different pomace extracts

	TPC	**FRAP**	**CUPRAC**	**ABTS$^{\bullet+}$ SC$_{max}$**	**ABTS$^{\bullet+}$ EC$_{20}$**
		apple			
FRAP	-0.473				
CUPRAC	0.537	0.489			
ABTS$^{\bullet+}$ SC$_{max}$	0.134	0.809	0.907		
DPPH$^{\bullet}$ SC$_{max}$	-0.605	0.988	0.347	0.708	
DPPH$^{\bullet}$ EC$_{20}$	0.856	-0.861	0.023		0.999**
ABTS$^{\bullet+}$ EC$_{20}$	0.874	-0.842	0.069		
ABTS$^{\bullet+}$ EC$_{50}$	0.057	-0.907	-0.811		
		Sour cherry			
FRAP	0.991				
CUPRAC	0.628	0.726			
ABTS$^{\bullet+}$ SC$_{max}$	0.984	0.990**	0.754		
DPPH$^{\bullet}$ SC$_{max}$	0.998**	0.997**	0.671	0.993	
DPPH$^{\bullet}$ EC$_{20}$	-0.750	-0.656	0.043		0.803
ABTS$^{\bullet+}$ EC$_{20}$	-0.996	-0.976	-0.559		
ABTS$^{\bullet+}$ EC$_{50}$	-0.899	-0.949**	-0.905		
		grape			
FRAP	0.999**				
CUPRAC	0.956	0.949			
ABTS$^{\bullet+}$ SC$_{max}$	0.999**	0.999**	0.960		
DPPH$^{\bullet}$ SC$_{max}$	0.739	0.724	0.904	0.750	
DPPH$^{\bullet}$ EC$_{20}$	0.510	0.530	0.235		0.992
ABTS$^{\bullet+}$ EC$_{20}$	-0.617	-0.634	-0.358		
ABTS$^{\bullet+}$ EC$_{50}$	-0.787	-0.773	-0.933		

*: significant at $p < 0.01$; **: significant at $p < 0.05$

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

Currently, there is a growing interest in recovery of antioxidants from fruit processing waste materials. Therefore, the estimation of antioxidant capacity is important. Researchers have expressed concerns about the reliability of existing antioxidant capacity assays due to poor correlations among different assay results and also the problem related to reproducibility of the results due to using different assay protocols. Therefore, a combination of a few assays must be carried out to provide a realistic assessment of the antioxidants.

This study showed the results of different assays to compare antioxidant capacities of dried apple, sour cherry and grape pomaces' extracts. UAE was applied with different solvents: ethanol, 50% aqueous ethanol and water for comparison. The antioxidant capacity assays were FRAP, CUPRAC, $DPPH^{\bullet}$, and $ABTS^{\bullet+}$ assays.

50% aqueous ethanol solvent provided the highest TPC in the extracts of sour cherry and grape pomace. On the other hand, for apple pomace samples, the highest TPC was obtained in water extracts. Results showed that antioxidant capacities of the fruit pomaces that were extracted with 50% aqueous ethanol solvent were highest and significantly different ($p<0.05$) from antioxidant capacities of pomace samples that were extracted with ethanol and water according to FRAP method. However, there is no significant difference between the antioxidant capacities of 50% aqueous ethanol and water extracts of apple and sour cherry pomaces according to CUPRAC method. Apple and sour cherry pomace 50 % aqueous ethanol extracts had the highest antioxidant capacity if compared with that of ethanol and water extracts according to $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays. Best antioxidant properties were found for grape pomace extracts but significant differences ($p>0.05$) were not observed for grape pomace 50 % aqueous ethanol,

water and ethanol extracts by using DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays. two different models were used for DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays. The logarithmic model had higher accuracy than exponential distribution model for the estimation of results.

Metal ion reducing capacity assays, FRAP and CUPRAC values were significantly ($p< 0.01$) correlated with TPC. Significant positive correlation between CUPRAC and FRAP assays was also obtained ($r= 0.885$) ($p<0.01$), therefore one of them can be selected for antioxidant capacity estimation. Low correlations were also obtained between DPPH$^{\bullet}$ EC_{20} and metal ion reducing capacity assays. The correlation was much better between ABTS$^{\bullet+}$ EC_{50} and FRAP assays especially for sour cherry pomace. The results of the study indicated that solvent type was important for the extraction of phenolic compounds. Also, only one antioxidant assay was not sufficient for the determination of antioxidant capacities of fruit pomace extracts.

In future studies, several standards instead of gallic acid can be used for the estimation of antioxidant capacity for FRAP and CUPRAC methods. Potential usage of antioxidants obtained from fruit pomace in the food industry can be investigated to prevent deterioration by oxidation during food processing and storage. For the selection of the most appropriate techniques, effects of different extraction techniquies such as microwave assisted extraction and high hydrostatic pressure assisted extraction with different ethanol:water mixture at different temperatures can be studied to compare mteal ion reducing antioxidant capacity assays and free radical scavenging assays

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APPENDICES

A. STATISTICAL ANALYSES

Table A.1 One-way Anova and Tukey’s comparison test for total phenolic content for apple samples which were extracted with 100% ethanol, 50% ethanol, water solvents

Total phenolic (mg GA/ml extract) of apple samples

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	3	Ethanol; 50% ethanol; Water

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	0,013524	0,006762	218,13	0,000
Error	6	0,000186	0,000031		
Total	8	0,013710			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0055678	98,64%	98,19%	96,95%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,08933	0,00208	(0,08147; 0,09720)
50% ethanol	3	0,07533	0,00569	(0,06747; 0,08320)
Water	3	0,16367	0,00751	(0,15580; 0,17153)

Pooled StDev = 0,00556776

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
Water	3	0,16367	A		
Ethanol	3	0,08933		B	
50% ethanol	3	0,07533			C

Means that do not share a letter are significantly different.

Total phenolic content (mg GA/ g dry pomace) of apple samples

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0,05$

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor 3 Ethanol; 50% ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	1,35470	0,677352	214,87	0,000
Error	6	0,01891	0,003152		
Total	8	1,37362			

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0,0561466 98,62% 98,16% 96,90%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,8953	0,0247	(0,8160; 0,9747)
50% ethanol	3	0,7500	0,0574	(0,6707; 0,8293)
Water	3	1,6360	0,0745	(1,5567; 1,7153)

Pooled StDev = 0,0561466

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
Water	3	1,6360	A		
Ethanol	3	0,8953		B	
50% ethanol	3	0,7500			C

Means that do not share a letter are significantly different.

Table A.2 One-way Anova and Tukey's comparison test for total phenolic content for sour cherry samples which were extracted with Ethanol, 50% ethanol, water solvents

Total phenolic content (mg GA/ml extract) sour cherry samples

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	**Levels**	**Values**
Factor	3	Ethanol; 50% ethanol; Water

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	0,10776	0,053878	10,87	0,010
Error	6	0,02973	0,004956		
Total	8	0,13749			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0,0703957	78,37%	71,17%	51,34%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,5133	0,1097	(0,4139; 0,6128)
50% ethanol	3	0,7533	0,0462	(0,6539; 0,8528)
Water	3	0,5300	0,0265	(0,4306; 0,6294)

Pooled StDev = 0,0703957

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
50% ethanol	3	0,7533	A	
Water	3	0,5300		B
Ethanol	3	0,5133		B

Means that do not share a letter are significantly different.

Total phenolic (mg GA/g dry pomace) of sour cherry samples

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	3	Ethanol; 50% ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	10,187	5,0936	10,31	0,011
Error	6	2,964	0,4939		
Total	8	13,151			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,702815	77,46%	69,95%	49,29%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	5,142	1,096	(4,149; 6,135)
50% ethanol	3	7,492	0,464	(6,499; 8,485)
Water	3	5,341	0,255	(4,348; 6,334)

Pooled StDev = 0,702815

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
50% ethanol	3	7,492	A	
Water	3	5,341		B
Ethanol	3	5,142		B

Means that do not share a letter are significantly different.

Table A.3 One-way Anova and Tukey's comparison test for total phenolic content for 3 different grape samples which were extracted with ethanol, 50% ethanol, water solvents

Total phenolic content (mg GA/ml extract) of grape samples

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	50% ethanol; Water; Ethanol

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	4,2455	2,12274	103,60	0,000
Error	6	0,1229	0,02049		
Total	8	4,3684			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,143139	97,19%	96,25%	93,67%

Means

Factor	N	Mean	StDev	95% CI
50% ethanol	3	2,8030	0,205	(2,601; 3,006)
Water	3	1,4233	0,1258	(1,2211; 1,6255)
Ethanol	3	1,2800	0,0600	(1,0778; 1,4822)

Pooled StDev = 0,143139

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
50% ethanol	3	2,8030	A	
Water	3	1,4233		B
Ethanol	3	1,2800		B

Means that do not share a letter are significantly different.

Total phenolic content (mg GA/g dry pomace) of grape samples

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0,05$

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	3	Ethanol; 50% ethanol; Water

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	423,14	211,568	106,54	0,000
Error	6	11,91	1,986		
Total	8	435,05			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
1,40919	97,26%	96,35%	93,84%

Means

Factor	**N**	**Mean**	**StDev**	**95% CI**
Ethanol	3	12,822	0,575	(10,832; 14,813)
50% ethanol	3	28,02	2,04	(26,03; 30,01)
Water	3	14,221	1,216	(12,231; 16,212)

Pooled StDev = 1,40919

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	**N**	**Mean**	**Grouping**	
50% ethanol	3	28,02	A	
Water	3	14,221		B
Ethanol	3	12,822		B

Means that do not share a letter are significantly different.

Table A.4 One-way Anova and Tukey's comparison test for antioxidant capacity of apple samples which were extracted with Ethanol, 50% ethanol, water solvents by using FRAP method

Antioxidant capacity (mg GAE/ml extract) of apple samples by FRAP method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor 3 Ethanol; %50 ethanol; Water

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	0,000550	0,000275	225,09	0,000
Error	6	0,000007	0,000001		
Total	8	0,000558			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0,0011055	98,68%	98,25%	97,04%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,012667	0,001155	(0,011105; 0,014228)
%50 ethanol	3	0,030667	0,001155	(0,029105; 0,032228)
Water	3	0,016000	0,001000	(0,014438; 0,017562)

Pooled StDev = 0,00110554

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
%50 ethanol	3	0,030667	A		
Water	3	0,016000		B	
Ethanol	3	0,012667			C

Means that do not share a letter are significantly different.

Antioxidant capacity (mg GAE/ mg dry pomace) of apple samples by FRAP method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	Ethanol; 50% ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0,056174	0,028087	327,86	0,000
Error	6	0,000514	0,000086		
Total	8	0,056688			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0092556	99,09%	98,79%	97,96%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,12733	0,01097	(0,11426; 0,14041)
50% ethanol	3	0,31033	0,01012	(0,29726; 0,32341)
Water	3	0,16433	0,00586	(0,15126; 0,17741)

Pooled StDev = 0,00925563

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
50% ethanol	3	0,31033	A		
Water	3	0,16433		B	
Ethanol	3	0,12733			C

Means that do not share a letter are significantly different.

Antioxidant capacity (mg GAE/mg TPC) of apple samples by FRAP method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	3	Ethanol; 50% ethanol; Water

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	0,173107	0,086553	754,10	0,000
Error	6	0,000689	0,000115		
Total	8	0,173796			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0,0107134	99,60%	99,47%	99,11%

Means

Factor	**N**	**Mean**	**StDev**	**95% CI**
Ethanol	3	0,14300	0,01212	(0,12786; 0,15814)
50% ethanol	3	0,41333	0,01358	(0,39820; 0,42847)
Water	3	0,10000	0,00361	(0,08486; 0,11514)

Pooled StDev = 0,0107134

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
50% ethanol	3	0,41333	A		
Ethanol	3	0,14300		B	
Water	3	0,10000			C

Means that do not share a letter are significantly different

Table A.5 One-way Anova and Tukey's comparison test for antioxidant capacity of sour cherry samples which were extracted with Ethanol, 50% ethanol, water solvents by using FRAP

Antioxidant capacity (mg GAE/ml extract) of sour cherry samples by FRAP method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0,05$

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	3	Ethanol; 50% ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0,042627	0,021313	86,02	0,000
Error	6	0,001487	0,000248		
Total	8	0,044114			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0157410	96,63%	95,51%	92,42%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,17800	0,00400	(0,15576; 0,20024)
50% ethanol	3	0,3377	0,0236	(0,3154; 0,3599)
Water	3	0,21100	0,01300	(0,18876; 0,23324)

Pooled StDev = 0,0157410

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
50% ethanol	3	0,3377	A	
Water	3	0,21100		B
Ethanol	3	0,17800		B

Means that do not share a letter are significantly different.

Antioxidant capacity (mg GAE mg /dry pomace) of sour cherry samples by FRAP method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	Levels	Values
Factor	3	Ethanol; %50 ethanol; Water

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	4,2556	2,12778	88,32	0,000
Error	6	0,1446	0,02409		
Total	8	4,4001			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0,155219	96,71%	95,62%	92,61%

Means

Factor	**N**	**Mean**	**StDev**	**95% CI**
Ethanol	3	1,7837	0,0431	(1,5644; 2,0029)
%50 ethanol	3	3,378	0,230	(3,158; 3,597)
Water	3	2,1093	0,1328	(1,8901; 2,3286)

Pooled StDev = 0,155219

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
%50 ethanol	3	3,378	A	
Water	3	2,1093		B
Ethanol	3	1,7837		B

Means that do not share a letter are significantly different.

Antioxidant capacity (mg GAE/mg TPC) of sour cherry samples by FRAP method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	**Levels**	**Values**
Factor	3	Ethanol; %50 ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0,020678	0,010339	18,35	0,003
Error	6	0,003380	0,000563		
Total	8	0,024058			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0,0237346	85,95%	81,27%	68,39%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,33333	0,00379	(0,29980; 0,36686)
%50 ethanol	3	0,4483	0,0314	(0,4148; 0,4819)
Water	3	0,4113	0,0263	(0,3778; 0,4449)

Pooled StDev = 0,0237346

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
%50 ethanol	3	0,4483	A	
Water	3	0,4113	A	
Ethanol	3	0,33333		B

Means that do not share a letter are significantly different.

Table A.6 One-way Anova and Tukey's comparison test for antioxidant capacity of grape samples which were extracted with Ethanol, 50% ethanol, water solvents by using FRAP method

Antioxidant capacity (mg GAE/ml extract) of grape samples by FRAP method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	3	Ethanol; %50 ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	1,42919	0,714595	209,50	0,000
Error	6	0,02047	0,003411		
Total	8	1,44965			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0584028	98,59%	98,12%	96,82%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,25900	0,01100	(0,17649; 0,34151)
%50 ethanol	3	1,1333	0,0811	(1,0508; 1,2158)
Water	3	0,3203	0,0595	(0,2378; 0,4028)

Pooled StDev = 0,0584028

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
%50 ethanol	3	1,1333	A	
Water	3	0,3203		B
Ethanol	3	0,25900		B

Means that do not share a letter are significantly different.

Antioxidant capacity (mg GAE mg /dry pomace) of grape samples by FRAP method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	**Levels**	**Values**
Factor	3	Ethanol; %50 ethanol; Water

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	143,417	71,7084	216,76	0,000
Error	6	1,985	0,3308		
Total	8	145,402			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0,575171	98,63%	98,18%	96,93%

Means

Factor	**N**	**Mean**	**StDev**	**95% CI**
Ethanol	3	2,5880	0,1171	(1,7754; 3,4006)
%50 ethanol	3	11,347	0,789	(10,534; 12,159)
Water	3	3,203	0,597	(2,390; 4,015)

Pooled StDev = 0,575171

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
%50 ethanol	3	11,347	A	
Water	3	3,203		B
Ethanol	3	2,5880		B

Means that do not share a letter are significantly different.

Antioxidant capacity (mg GAE/mg TPC) of grape samples by FRAP method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	Ethanol; %50 ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0,073733	0,036866	42,40	0,000
Error	6	0,005217	0,000870		
Total	8	0,078950			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0294882	93,39%	91,19%	85,13%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,20233	0,00902	(0,16067; 0,24399)
%50 ethanol	3	0,4047	0,0284	(0,3630; 0,4463)
Water	3	0,2250	0,0415	(0,1833; 0,2667)

Pooled StDev = 0,0294882

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
%50 ethanol	3	0,4047	A	
Water	3	0,2250		B
Ethanol	3	0,20233		B

Means that do not share a letter are significantly different.

Table A.7 One-way Anova and Tukey's comparison test for antioxidant capacity of apple samples which were extracted with Ethanol, 50% ethanol, water solvents by using CUPRAC method

Antioxidant capacity (mg GAE/mg extract) of apple samples by CUPRAC method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0,05$

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor 3 Ethanol; 50% ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0,001500	0,000750	23,36	0,001
Error	6	0,000193	0,000032		
Total	8	0,001693			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0056667	88,62%	84,83%	74,39%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,03300	0,00361	(0,02499; 0,04101)
50% ethanol	3	0,05667	0,00208	(0,04866; 0,06467)
Water	3	0,06300	0,00889	(0,05499; 0,07101)

Pooled StDev = 0,00566667

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
Water	3	0,06300	A	
50% ethanol	3	0,05667	A	
Ethanol	3	0,03300		B

Means that do not share a letter are significantly different.

Antioxidant capacity (mg GAE/mg dry pomace) of apple samples by CUPRAC method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	3	Ethanol; %50 ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0,15099	0,075497	23,01	0,002
Error	6	0,01969	0,003281		
Total	8	0,17068			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0572790	88,47%	84,62%	74,05%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,3303	0,0348	(0,2494; 0,4113)
%50 ethanol	3	0,5670	0,0225	(0,4861; 0,6479)
Water	3	0,6317	0,0901	(0,5507; 0,7126)

Pooled StDev = 0,0572790

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
Water	3	0,6317	A	
%50 ethanol	3	0,5670	A	
Ethanol	3	0,3303		B

Means that do not share a letter are significantly different.

Antioxidant capacity (mg GAE/mg TPC) of apple samples by CUPRAC method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	Ethanol; %50 ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0,29214	0,146071	86,01	0,000
Error	6	0,01019	0,001698		
Total	8	0,30233			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0412108	96,63%	95,51%	92,42%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,3710	0,0380	(0,3128; 0,4292)
%50 ethanol	3	0,7600	0,0274	(0,7018; 0,8182)
Water	3	0,3850	0,0539	(0,3268; 0,4432)

Pooled StDev = 0,0412108

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
%50 ethanol	3	0,7600	A	
Water	3	0,3850		B
Ethanol	3	0,3710		B

Means that do not share a letter are significantly different

Table A.8 One-way Anova and Tukey's comparison test for antioxidant capacity of sour cherry samples which were extracted with Ethanol, 50% ethanol, water solvents by using CUPRAC method

Antioxidant capacity (mg GAE/ml extract) of sour cherry samples by CUPRAC method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	3	Ethanol; 50% ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0,07219	0,036094	12,31	0,008
Error	6	0,01760	0,002933		
Total	8	0,08979			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0541592	80,40%	73,87%	55,90%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,3737	0,0832	(0,2972; 0,4502)
50% ethanol	3	0,5730	0,0297	(0,4965; 0,6495)
Water	3	0,5527	0,0316	(0,4762; 0,6292)

Pooled StDev = 0,0541592

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
50% ethanol	3	0,5730	A	
Water	3	0,5527	A	
Ethanol	3	0,3737		B

Means that do not share a letter are significantly different.

Antioxidant capacity (mg GAE/ mg dry pomace) of sour cherry samples by CUPRAC method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	3	Ethanol; %50 ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	7,216	3,6080	12,40	0,007
Error	6	1,745	0,2909		
Total	8	8,961			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,539340	80,52%	74,03%	56,18%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	3,738	0,829	(2,976; 4,500)
%50 ethanol	3	5,732	0,296	(4,970; 6,494)
Water	3	5,527	0,314	(4,765; 6,289)

Pooled StDev = 0,539340

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
%50 ethanol	3	5,732	A	
Water	3	5,527	A	
Ethanol	3	3,738		B

Means that do not share a letter are significantly different.

Antioxidant capacity (mg GAE/mg TPC) of sour cherry samples by CUPRAC method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	3	Ethanol; %50 ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0,24138	0,120688	12,16	0,008
Error	6	0,05954	0,009924		
Total	8	0,30092			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0,0996193	80,21%	73,62%	55,48%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,7050	0,1565	(0,5643; 0,8457)
%50 ethanol	3	0,7610	0,0394	(0,6203; 0,9017)
Water	3	1,0770	0,0610	(0,9363; 1,2177)

Pooled StDev = 0,0996193

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
Water	3	1,0770	A	
%50 ethanol	3	0,7610		B
Ethanol	3	0,7050		B

Means that do not share a letter are significantly different.

Table A.9 One-way Anova and Tukey's comparison test for antioxidant capacity of grape samples which were extracted with Ethanol, 50% ethanol, water solvents by using CUPRAC method

Antioxidant capacity (mg GAE/ml extract) of grape samples by CUPRAC method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0,05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	Ethanol; 50% ethanol; Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0,50058	0,250288	108,96	0,000
Error	6	0,01378	0,002297		
Total	8	0,51436			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0479270	97,32%	96,43%	93,97%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,6993	0,0331	(0,6316; 0,7670)
50 % ethanol	3	1,2713	0,0706	(1,2036; 1,3390)
Water	3	0,9153	0,0286	(0,8476; 0,9830)

Pooled StDev = 0,0479270

<u>Tukey Pairwise Comparisons</u>

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
50% ethanol	3	1,2713	A		
Water	3	0,9153		B	
Ethanol	3	0,6993			C

Means that do not share a letter are significantly different.

Antioxidant capacity (mg GAE/mg dry pomace) of grape samples by CUPRAC method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	3	Ethanol; %50 ethanol; Water

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	44,1448	22,0724	308,96	0,000
Error	6	0,4286	0,0714		
Total	8	44,5735			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0,267285	99,04%	98,72%	97,84%

Means

Factor	**N**	**Mean**	**StDev**	**95% CI**
Ethanol	3	6,996	0,331	(6,618; 7,373)
%50 ethanol	3	12,3850	0,1585	(12,0074; 12,7626)
Water	3	9,153	0,282	(8,775; 9,531)

Pooled StDev = 0,267285

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	**N**	**Mean**	**Grouping**		
%50 ethanol	3	12,3850	A		
Water	3	9,153		B	
Ethanol	3	6,996			C

Means that do not share a letter are significantly different.

Antioxidant capacity (mg GAE/mg TPC) of grape samples by CUPRAC method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values

Factor	3	Ethanol; %50 ethanol; Water

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	0,053965	0,026982	46,77	0,000
Error	6	0,003461	0,000577		
Total	8	0,057426			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0,0240185	93,97%	91,96%	86,44%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0,5467	0,0265	(0,5127; 0,5806)
%50 ethanol	3	0,4533	0,0250	(0,4194; 0,4873)
Water	3	0,6430	0,0201	(0,6091; 0,6769)

Pooled StDev = 0,0240185

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
Water	3	0,6430	A		
Ethanol	3	0,5467		B	
%50 ethanol	3	0,4533			C

Means that do not share a letter are significantly different.

Table A.10 One-way Anova and Tukey's comparison test for EC_{20} values of apple samples (mg GAE/ml extract) which were extracted with Ethanol, 50% ethanol, water solvents by DPPH method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	50% ethanol, Water, Ethanol

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.004562	0.002281	128.51	0.000
Error	6	0.000106	0.000018		
Total	8	0.004668			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0042128	97.72%	96.96%	94.87%

Means

Factor	N	Mean	StDev	95% CI
50% ethanol	3	0.006770	0.001160	(0.000818, 0.012722)
Water	3	0.061133	0.001026	(0.055182, 0.067085)
Ethanol	3	0.04197	0.00713	(0.03602, 0.04792)

Pooled StDev = 0.00421281

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
Water	3	0.061133	A		
Ethanol	3	0.04197		B	
50% ethanol	3	0.006770			C

Means that do not share a letter are significantly different.

Table A.11 Table B One-way Anova and Tukey's comparison test for EC_{20} values of sour cherry samples(mg GAE/ml extract)which were extracted with Ethanol, 50% ethanol, water solvents by DPPH method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	**Levels**	**Values**
Factor	3	50% ethanol, Water, Ethanol

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	0.000033	0.000016	77.29	0.000
Error	6	0.000001	0.000000		
Total	8	0.000034			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0.0004607	96.26%	95.02%	91.59%

Means

Factor	**N**	**Mean**	**StDev**	**95% CI**
50% ethanol	3	0.012533	0.000737	(0.011883, 0.013184)
Water	3	0.017167	0.000153	(0.016516, 0.017817)
Ethanol	3	0.014300	0.000265	(0.013649, 0.014951)

Pooled StDev = 0.000460676

<u>Tukey Pairwise Comparisons</u>

Grouping Information Using the Tukey Method and 95% Confidence

Factor	**N**	**Mean**	**Grouping**		
Water	3	0.017167	A		
Ethanol	3	0.014300		B	
50% ethanol	3	0.012533			C

Means that do not share a letter are significantly different.

Table A.12 One-way Anova and Tukey's comparison test for EC_{50} values of grape samples (mg GAE/ml extract) which were extracted with Ethanol, 50% ethanol, water solvents by DPPH method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	**Levels**	**Values**
Factor	2	50% ethanol, Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	1	0.000156	0.000156	22.17	0.009
Error	4	0.000028	0.000007		
Total	5	0.000184			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0026503	84.72%	80.90%	65.62%

Means

Factor	N	Mean	StDev	95% CI
50% ethanol	3	0.034657	0.000484	(0.030408, 0.038905)
Water	3	0.02447	0.00372	(0.02022, 0.02872)

Pooled StDev = 0.00265028

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
50% ethanol	3	0.034657	A	
Water	3	0.02447		B

Means that do not share a letter are significantly different.

Table A.13 One-way Anova and Tukey's comparison test for EC_{20} values of grape samples(mg GAE/ml extract)which were extracted with Ethanol, 50% ethanol, water solvents by DPPH method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	**Levels**	**Values**
Factor	3	50% ethanol, Water, Ethanol

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	0.000039	0.000020	2.07	0.207
Error	6	0.000057	0.000010		
Total	8	0.000097			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0.0030873	40.83%	21.10%	0.00%

Means

Factor	N	Mean	StDev	95% CI
50% ethanol	3	0.01620	0.00290	(0.01184, 0.02056)
Water	3	0.011543	0.001631	(0.007182, 0.015905)
Ethanol	3	0.01573	0.00419	(0.01137, 0.02009)

Pooled StDev = 0.00308731

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
50% ethanol	3	0.01620	A
Ethanol	3	0.01573	A
Water	3	0.011543	A

Means that do not share a letter are significantly different.

Table A.14 One-way Anova and Tukey's comparison test for EC_{50} values of apple samples (mg GAE/ml extract) which were extracted with Ethanol, 50% ethanol, water solvents by ABTS method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	Ethanol, 50% ethanol, Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.000053	0.000027	479.18	0.000
Error	6	0.000000	0.000000		
Total	8	0.000054			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0002357	99.38%	99.17%	98.60%

Means

Factor	N	Mean	StDev	95% CI
Ethanol	3	0.007600	0.000300	(0.007267, 0.007933)
50% ethanol	3	0.001667	0.000153	(0.001334, 0.002000)
Water	3	0.004167	0.000231	(0.003834, 0.004500)

Pooled StDev = 0.000235702

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
Ethanol	3	0.007600	A		
Water	3	0.004167		B	
50% ethanol	3	0.001667			C

Means that do not share a letter are significantly different.

Table A.15 One-way Anova and Tukey's comparison test for EC_{20} values of apple samples (mg GAE/ml extract) which were extracted with Ethanol, 50% ethanol, water solvents by ABTS method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	**Levels**	**Values**
Factor	3	Ethanol, 50% ethanol, Water

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	0.000001	0.000001	233.50	0.000
Error	6	0.000000	0.000000		
Total	8	0.000001			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0.0000525	98.73%	98.31%	97.15%

Means

Factor	**N**	**Mean**	**StDev**	**95% CI**
Ethanol	3	0.001247	0.000050	(0.001173, 0.001321)
50% ethanol	3	0.000657	0.000055	(0.000583, 0.000731)
Water	3	0.001570	0.000052	(0.001496, 0.001644)

Pooled StDev = 0.0000524934

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
Water	3	0.001570	A		
Ethanol	3	0.001247		B	
50% ethanol	3	0.000657			C

Means that do not share a letter are significantly different.

Table A.16 One-way Anova and Tukey's comparison test for EC_{50} values of sour cherry samples (mg GAE/ml extract) which were extracted with Ethanol, 50% ethanol, water solvents by ABTS method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	**Levels**	**Values**
Factor	2	50% ethanol, Water

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	1	0.000011	0.000011	1468.81	0.000
Error	4	0.000000	0.000000		
Total	5	0.000011			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0000855	99.73%	99.66%	99.39%

Means

Factor	N	Mean	StDev	95% CI
50% ethanol	3	0.003123	0.000068	(0.002986, 0.003260)
Water	3	0.005800	0.000100	(0.005663, 0.005937)

Pooled StDev = 0.0000855375

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
Water	3	0.005800	A	
50% ethanol	3	0.003123		B

Means that do not share a letter are significantly different.

Table A.17 One-way Anova and Tukey's comparison test for EC_{20} values of sour cherry samples (mg GAE/ml extract) which were extracted with Ethanol, 50% ethanol, water solvents by ABTS method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	**Levels**	**Values**
Factor	3	50% ethanol, Water, Ethanol

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	0.000000	0.000000	222.19	0.000
Error	6	0.000000	0.000000		
Total	8	0.000000			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0.0000276	98.67%	98.22%	97.00%

Means

Factor	**N**	**Mean**	**StDev**	**95% CI**
50% ethanol	3	0.001136	0.000004	(0.001097, 0.001175)
Water	3	0.001555	0.000040	(0.001516, 0.001594)
Ethanol	3	0.001540	0.000026	(0.001501, 0.001579)

Pooled StDev = 0.0000276426

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	**N**	**Mean**	**Grouping**	
Water	3	0.001555	A	
Ethanol	3	0.001540	A	
50% ethanol	3	0.001136		B

Means that do not share a letter are significantly different.

Table A.18 One-way Anova and Tukey's comparison test for EC_{50} values of grape sapmles (mg GAE/ml extract) which were extracted with Ethanol, 50% ethanol, water solvents by ABTS method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	**Levels**	**Values**
Factor	3	50% ethanol, Water, Ethanol

Analysis of Variance

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Factor	2	0.000000	0.000000	30.79	0.001
Error	6	0.000000	0.000000		
Total	8	0.000000			

Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0.0000821	91.12%	88.16%	80.03%

Means

Factor	N	Mean	StDev	95% CI
50% ethanol	3	0.002063	0.000076	(0.001947, 0.002179)
Water	3	0.002183	0.000116	(0.002067, 0.002299)
Ethanol	3	0.002567	0.000032	(0.002451, 0.002683)

Pooled StDev = 0.0000820569

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
Ethanol	3	0.002567	A	
Water	3	0.002183		B
50% ethanol	3	0.002063		B

Means that do not share a letter are significantly different.

Table A.19 One-way Anova and Tukey's comparison test for EC_{20} values of grape sapmles (mg GAE/ml extract) which were extracted with Ethanol, 50% ethanol, water solvents by ABTS method

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	50% ethanol, Water, ethanol

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.000000	0.000000	1.58	0.282
Error	6	0.000000	0.000000		
Total	8	0.000000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0002312	34.44%	12.58%	0.00%

Means

Factor	N	Mean	StDev	95% CI
50% ethanol	3	0.000670	0.000010	(0.000343, 0.000997)
Water	3	0.000990	0.000400	(0.000663, 0.001317)
ethanol	3	0.000744	0.000003	(0.000417, 0.001070)

Pooled StDev = 0.000231236

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
Water	3	0.000990	A
ethanol	3	0.000744	A
50% ethanol	3	0.000670	A

Means that do not share a letter are significantly different.