NUTRACEUTICAL ASSESSMENT OF GEORGIA-GROWN POMEGRANATE JUICE

by

DHIVYALAKSHMI RAJASEKAR

(Under the Direction of Casimir C. Akoh)

ABSTRACT

Pomegranate (*Punica granatum* L.) juice is widely known for its potential health benefits. The juice was extracted using two methods, namely blender and mechanical press. Fourteen Georgia-grown pomegranate cultivars, harvested in 2009 were analyzed for juice yield, antioxidant capacity (Ferric Reducing Antioxidant Power, FRAP; Trolox Equivalent Antioxidant Capacity, TEAC; Oxygen Radical Absorbance Capacity, ORAC), total anthocyanins, total polyphenols, major sugars, organic acids, and individual phenolic compounds. Citric acid was the predominant acid, and glucose and fructose were the major sugars found. Cultivar Cranberry had the highest significant ($p \leq 0.05$) total polyphenols and antioxidant capacity. Also, fifteen Georgia-grown pomegranates harvested in 2010 were investigated for their physico-chemical characteristics, juice yield, total anthocyanins, antioxidant capacity, total polyphenols, and individual anthocyanins. The major anthocyanin found was delphinidin-3-glucoside. Cultivar Kaj-acik-anor had the highest significant ($p \leq 0.05$) total anthocyanin. Significant ($p \leq 0.05$) differences among cultivars were observed. Positive correlations were found between total polyphenols and antioxidant capacity method, FRAP. Overall, blender was an

efficient method of juice extraction, mainly due to high juice yield, total polyphenols, and antioxidant capacity.

INDEX WORDS: Pomegranate (*Punica granatum* L.) juice, extraction methods, yield, total polyphenols, antioxidant capacity, total anthocyanins.

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DEDICATION

To Sadhguru Jaggi Vasudev, my parents and brother

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CHAPTER 1

INTRODUCTION

The consumption of red fruits has increased tremendously in recent times as they are a rich source of antioxidant phenolics and anthocyanins. The juice of pomegranate fruit (*Punica granatum* L.) contains higher levels of antioxidants compared to other fruit juices and beverages (Seeram, Aviram, Zhang, Henning, Feng, Dreher et al., 2008). Gil, Tomas-Barberan, Hess-Pierce, & Kader (2000) reported that commercial pomegranate juice has three times higher antioxidant capacity compared to red wine and green tea. Clinical research studies have evaluated the health benefits of pomegranate juice. They suggested that consumption of pomegranate juice helped in lowering LDL and cholesterol levels (Aviram & Dornfeld, 2001), increased prostate specific antigen, PSA (Pantuck, Leppert, Zomorodian, Aronson, Wong, Barnard et al., 2006), protection against heart disease (Sumner, Elliott-Eller, Weidner, Daubenmier, Chew, Marlin et al., 2005), Alzheimer's disease (Singh, Arseneault, Sanderson, Morthy, & Ramassamy, 2008), cancer (Seeram, Aronson, Zhang, Henning, Moro, Lee et al., 2007), improved sperm quality (Türk, Sönmez, Aydin, Yüce, Gür, Yüksel et al., 2008), and erectile dysfunction in male patients (Forest, Padma-Nathan, & Liker, 2007).

The pomegranate fruit is round in shape with an outer leathery skin or rind. They are generally yellow and may be overlaid with light to deep pink or rich red. The arils or the juice sacs are the edible part of the fruit. Their colors vary from yellow to deep red and typically consist of 80% juice and 20% seed by weight. The edible part can

be consumed fresh or used for the preparation of fresh juice, canned beverages, jelly, jam, paste, and also as a flavoring and coloring agent in beverage products. The red color of pomegranate juice is due to the presence of anthocyanins namely cyanidin, delphinidin, and pelargonidin. They also consist of some phenolics and tannins like punicalin, pedunculagin, punicalagin, and ellagic acid, which can serve as primary antioxidative phenolics (Kulkarni & Aradhya, 2005). Citric and malic acids are the major organic acids, while glucose and fructose are the major sugars found in the juice. Organic acid profile helps in characterization of flavor, freshness or spoilage of the juice. The sugar profiles are important to detect adulteration of fruit juices (Tezcan, Gültekin-Özgüven, Diken, Özçelik, & Erim, 2009).

The increased attention gained by pomegranate juice due to its varied potential health benefits has resulted in its increased demand in the Western world. Therefore, pomegranate growth and production has seen a significant increase in many regions. Pomegranate cultivation is adapted to Mediterranean type climate having semi-arid mild-temperature to subtropical climates with hot summers and cool winters. They are widely grown in countries like Iran, India, Turkey, China, Japan, Afghanistan, and United States (Stover & Mercure, 2007). The local cultivars differ distinctively in their aril colors and flavor profiles. It has been reported that the antioxidant capacity of pomegranate juice depends on cultivar, growing region, climate, maturity, cultural practice, and the method used to obtain the juice (Çam, Hışıl, & Durmaz, 2009). Also, cultivars may also influence physicochemical properties like juice percentage, dry matter, pH, total soluble solids (TSS), total sugars, titratable acidity (TA), total phenolics, and anthocyanins. With

these parameters, the quality of a cultivar can be defined and the consumer would be able to select a more nutritional fruit (Tehranifar, Zarei, Esfandiyari, & Nemati, 2010).

The commercial production of pomegranate in Georgia is at its early stages. The leading causes of death in Georgia are cancer and cardiovascular diseases accounting for a quarter and one third of all deaths in the state, respectively. A detailed cultivar characterization would enable the pomegranate growers to successfully identify a potential cultivar for commercial production.

The current thesis is divided into six chapters. The first chapter is an introduction to the research with the overall objectives. The second chapter consists of the literature review covering topics such as pomegranate production, cultivation, composition, oxidative stress, antioxidants, methods used to determine antioxidative capacities, phenolics, anthocyanins, and tannins.

The third chapter is the characterization of aril juice of fourteen pomegranate cultivars harvested in 2009, extracted using blender and mechanical press. The cultivars include White Don Wade, Turk Don Wade, Haku-botan, Don Sumner South Tree, Don Sumner North Tree, Mejhos, Salavatski, Kaj-acik-anor, Nikitski ranni, Afganski, Entek Habi Saveh, Eve, Cranberry, and Cloud. They were analyzed for juice yield, dry matter, total polyphenols, antioxidant capacity by FRAP, TEAC, and ORAC, total monomeric anthocyanins, major organic acids, sugars, and major individual phenolic compounds.

The fourth chapter is the physico-chemical characterization of aril juice of fifteen pomegranate cultivars harvested in 2010, extracted using blender and mechanical press. The cultivars include Kaj-acik-anor, Rose, Don Sumner South Tree, Don Sumner North Tree, King, Crab, Thompson, Entek Habi Saveh, Afganski, Nikitski ranni,

Fleshman, Haku-botan, Salavatski, Cranberry, and Pink. They were analyzed for juice yield, pH, total soluble solids (TSS), titratable acidity (TA), formol number, color values, total polyphenols, antioxidant capacity by FRAP, TEAC, and ORAC, total monomeric anthocyanins by pH differential method, and individual anthocyanins by RP-HPLC.

The fifth chapter is the comparison of nine cultivars namely Don Sumner South Tree, Don Sumner North Tree, Haku-botan, Salavatski, Kaj-acik-anor, Nikitski ranni, Afganski, Entek Habi Saveh, and Cranberry, between two different years of harvest (2009 & 2010). The aril juice was extracted using blender and mechanical press methods, and analyzed for juice yield, total polyphenols, antioxidant capacity by FRAP, TEAC, ORAC, and total monomeric anthocyanins. The last chapter includes the overall conclusions of the studies carried out.

The objectives of this research are:

1) To determine and compare the juice yield, total polyphenols, antioxidant capacity, and total anthocyanins among different cultivars.
2) To determine the major organic acids, phenolic compounds profile, and the major individual sugars.
3) To determine the physico-chemical properties of aril juice, their color values and individual anthocyanin profile.
4) To compare the yield, antioxidant capacity, total phenolics, and anthocyanins between harvest years 2009 and 2010.

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CHAPTER 2

LITERATURE REVIEW

Pomegranate

Pomegranate (*Punica granatum* L.) is considered to be one of the new super foods by consumers in the United States, mainly due to its various health benefits. For thousands of years, this fruit has been consumed and used for its medicinal properties in the Middle East. Recently, gained popularity of the fruit in the United Sates has led to widespread introduction of pomegranate products, including 100% juices, pomegranate-containing beverages, liquid and powdered polyphenolic extracts of pomegranate plant parts like leaves, flowers, arils, and peel, pomegranate seed oil, and skin care products with pomegranate extracts. The potential use of pomegranate may be as an antioxidant, anti-inflammatory, antiviral, antibacterial, and antifungal agent, which contributes to its health beneficial properties. They are also known to possess anticancer properties, improve cardiovascular health, prevent diabetes and rheumatoid arthritis, improve male virility and erectile function, nourishment of the skin with antiwrinkle effects, and protect against Alzheimer's disease (Johanningsmeier & Harris, 2011).

Cultivation and production

Pomegranate was greatly appreciated in the Arabic and Hebrew cultures, as they were called "fruit of paradise." Pomegranate is an ancient fruit and with its cultivation dating back to 3000 BC in Persia (Iran) (Anarinco, 2006). Pomegranates were brought to modern-day Tunisia and Egypt by Phoenicians around 2000 BC and around the same time it was introduced in western Turkey and Greece. Around 100 BC, the fruit reached

China. It was cultivated in Central and southern India by 800 CE (Morton, 1987). The Spanish cultivars introduced pomegranate to Central America, Mexico, and South America in the 1500 and 1600s (LaRue, 1980). In the early 1700s, clear evidence of pomegranate cultivation in the United States was seen in Spanish Florida and English Georgia. It spread to the West Coast in the 1770s and is widely grown in Califiornia (Morton, 1987).

The production of pomegranates in India is more than 100,000 ha, and in Turkey 56,000 tons/year was produced in 1997 (Gozlekci & Kaynak, 2000). The largest western European pomegranate producer is Spain, around 3000 ha, with increased production due to the high market prices (Costa & Melgarejo, 2000). Commercial pomegranate in the United States is grown in the San Joaquin Valley on 5600 ha, with the predominant cultivar being 'Wonderful.'

The fruit

Pomegranate is one of the oldest known edible fruits, with a leathery rind (husk), enclosing the arils which contain seeds. The different parts of a pomegranate fruit are shown in Fig 2.1. The arils are the juice sacs composed of epidermal cells, and range from deep red to colorless depending on the cultivar type. Seed softness is influenced by sclerenchyma tissues present in the seed. The number of locules, arils, and seeds differ, and can go as high as 1300 per fruit (Stover & Mercure, 2007). The edible part of the pomegranate fruit (50%) is primarily composed of 40% arils and 10% seeds. Generally, the composition of arils includes 85% water, 10% total sugars, mainly glucose and fructose, 1.5% pectin, organic acids like ascorbic, citric, and malic acid, and bioactive compounds such as phenolics and flavonoids, mainly anthocyanins (Aviram, Dornfeld,

Rosenblat, Volkova, Kaplan, Coleman et al., 2000). The husk consists of the pericarp, which provides a cuticle layer and fibrous mat, and mesocarp (albedo), the spongy tissue and inner fruit wall to which arils are attached (Fig 2.1).

Cultivars

A large variety of pomegranate cultivars are found all over the world. The important characteristics of a cultivar are fruit size, husk color (yellow to purple, pink, and red), aril color (white to red), hardness of the seed, maturity, juice content, acidity, sweetness, and astringency (Stover et al., 2007). The cultivar grown widely in the United States is 'Wonderful' which has a deep color in both husk and juice, rich flavor, high juice yield, and acceptable levels of acidity and astringency. It must also be resistant to fruit cracking during rainfall on a mature fruit (Karp, 2006). It is also grown in Western Europe, Chile, and Israel (Sepulveda, Galleti, Saenz, & Tapia, 2000). Cultivars Grananda, Early Wonderful, and Early Foothill are the other commercial ones grown in the United States.

In Spain, the cultivars Mollar de Elche and Valenciana are the most widely marketed ones. Small fruit sizes, low yield, average to poor internal quality are some of the characteristics of cultivar Valenciana. It is harvested early (August) with almost no sun damage and pest attacks. Cultivar Mollar is harvested at the end of September with more sun damage, high yield, high internal fruit quality, big size, and greater consumer acceptance (Costa et al., 2000).

Pomegranate germplasm collections of local cultivars have been established in Mediterranean countries like Spain, Morocco, Tunisia, Greece, Turkey, and Egypt (Mars, 2000). More than 200 accessions, including Turkmenistan collections are there in the

U.S. National Clonal Germplasm Repository, in Davis, CA. The largest germplasm collection with more than 1000 accessions is present in the Turkmenistan Experimental Station of Plant Genetic Resources (Levin, 1995).

Climatic conditions

Pomegranates are grown in Mediterranean type climates having cool winters and hot summers with semi-arid mild-temperature to subtropical climates. Dry summer climates are suited for commercial production of pomegranates. They are extremely tolerant to drought and salinity, and can be grown in various soil conditions. For the new planted trees to thrive well, enough moisture is needed. In California, new trees are planted in late winter to spring, as the soil would have high moisture levels from the winter rain (Stover et al., 2007). In Georgia, the flower bloom occurs in April, and the fruits are harvested in September. Temperatures above 85 °F are required for atleast 120 days a year with six hours of direct sunlight for production of quality fruits. In south Georgia, pomegranates are planted on a raised bed atleast 4 feet wide and 6 to 12 inches in height. Pomegranate orchards in Georgia are planted in 20 – 30 acres. For pomegranate production in Georgia, cultivar Wonderful does not grow very well here, due to its low chilling tolerance during extreme winter conditions. Humidity conditions in Georgia play an important role in pomegranate cultivation. For early blooming cultivars, increased humidity levels during bloom and fruit set and low humidity levels in mid to late spring would greatly benefit in the development of good quality fruits (MacLean, Martino, Scherm, & Horton, 2011). Having adequate moisture levels throughout the growing season is important as it would help in the proper development, production, and reduce its splitting (LaRue, 1980). The tree would produce a few fruits in the second or third year

after propagation, but commercial production is seen only in 5 to 6 years. Karp (2006) reported that California commercial pomegranate orchards would produce mature yields of 33 $t.ha^{-1}$. Generally, pomegranate orchards produce 0.2 to 0.5 kg N/tree per year, harvested either in fall or winter.

Processing

The processing method for pomegranates depends on its use. Fresh or processed arils, jams, jellies, juices, teas, beverages, concentrated syrups, and liquors are some of the common ways of utilizing the fruit. The arils are dried in the sun for 15 days in India, and sold as a spice called 'anardana,' which helps with digestion and mouthfeel (Kingsly, Singh, Manikantan, & Jain, 2006). The byproduct of pomegranate juice production is rich in fiber and used as cattle feed.

The fresh fruit can be consumed by cutting the fruit into equal halves, lifting out the clusters of juice sacs/arils from the rind. For homemade production, the arils are removed from the rind, and then pressed in cheese cloth. Juice can also be prepared with blender followed by straining to remove the seeds. On a lab scale, juice extraction involves cutting the fruit, separation of arils, and extraction of the juice with blender, hand press, electric juice centrifuge, electric lemon squeezer, or a juice extractor (MacLean et al., 2011; Gil, Tomás-Bareberán, Hess-Pierce, Holcroft, & Kader, 2000; Miguel, Dandlen, Antunes, Neves, & Martins, 2004; Tzulker, Glazer, Bar-Ilan, Holland, Aviram, & Amir, 2007). The arils can also be minimally processed by washing with chlorinated water and antioxidant solution to lower the microbial growth and improve shelf life. Controlled atmosphere packaging of arils is done using polymeric, perforated

polyethylene or semi-permeable film. The semi-permeable film allowed storage for 14 days at 4 °C (López-Rubira, Conesa, Allende, & Artés, 2005).

Industrial production of POM Wonderful® involves crushing of the whole fruit with the appropriate hydrostatic pressure, resulting in the release of juice from arils and also extracting the water soluble ellagitannins from the rind into the juice. In some other processes, membrane press is used in order to reduce contamination from bitter compounds like tannins and seeds. Several filters such as vacuum rotating filters, plate filters and ultra filtration is used for filtration, followed by evaporation to produce clear and concentrated juices which are sterilized and bottled. Storage at – 20 °C allowed the juice to be stable for six months (Weusthuis, 2009).

Functional properties

Institute of Food Technologists (IFT, 2009) has defined functional foods as "foods and food components that provide a health benefit beyond basic nutrition (for the intended population). These substances provide essential nutrients often beyond quantities necessary for normal maintenance, growth, and development, and/ or other biologically active components that impart health benefits or desirable physiological effects."Official regulations do not exist for functional foods by FDA in the United States. Modification or elimination of one or more of the ingredients may be considered a functional food. They may help in the maintenance of health or well being, or reduce the risk of suffering a given illness (Pérez-Alvarez, Sayas-Barberá, & Fernández-López, 2003). When developing a functional product, one must keep in mind, consumer expectations, which include good taste, wholesomeness, and high nutritional values (García-segovia, Andres-Bello, & Martinez-Monzo, 2007).

Pomegranate fruit may be considered a functional food mainly due to the presence of bioactive components, which possess various functional properties and health benefits as shown in Fig. 2.2. They are known to improve cardiovascular health (Davidson, Maki, Dicklin, Feinstein, Witchger, Bell et al., 2009), and possess antioxidant (Çam, Hışıl, & Durmaz, 2009), anti-inflammatory (Lee, Chen, Liang, & Wanga, 2010), antimicrobial (Duman, Ozgen, Dayisoylu, Erbil, & Durgac, 2009), antitumoral (Hamad & Al-Momene, 2009), and antidiabetic properties (Xu, Zhu, Kim, Yamahara, & Li, 2009). They also aid in the prevention of Alzheimer's disease (Singh, Arseneault, Sanderson, Morthy, & Ramassamy, 2008), improve sperm quality (Türk, Sönmez, Aydin, Yüce, Gür, Yüksel et al., 2008) and erectile dysfunction in male patients (Forest, Padma-Nathan, & Liker) and improve oral (DiSilvestro, DiSilvestro, & DiSilvestro, 2009) and skin (Aslam, Lansky, & Varani, 2006) health.

Composition of pomegranate

The most popular cultivar "Wonderful," grown in California has a dark purple-red skin color with a shiny outer appearance. The juice from these fruits have a dark crimson color with a better flavor, mainly due to the increased levels of sugars and acid content (Adsule & Patil, 1995). Their seed sizes are small and tender, and the rind is not too thick (Kader, Chordas, & Elyatem, 1984). Spanish cultivar "Mollar" has white to pink arils which are sweeter when compared to purple or dark colored arils, due to the elevated levels of organic acid present (Gil, Sanchez, Marin, & Artes, 1996). The predominant acid was citric acid, with titratable acidity values of 1 to 2% reported based on fresh weight. The major sugars found were glucose and fructose, which are in the range of 14 to 17% based on fresh weight (Kader et al., 1984). The phenolic compounds

are ellagic acid derivatives and hydrolyzable tannins such as punicalin and punicalagin (Gil et al., 2000). Commonly found anthocyanins in pomegranate juice are the 3-glucosides and 3,5-glucosides of delphinidin, cyanidin, and pelargonidin (Alighourchi, Barzegar, & Abbasi, 2008; Miguel et al., 2004) (Fig. 2.3). Gil et al.(2000). reported positive correlations between total phenolics and antioxidant capacity of the pomegranate juice.

As a pomegranate fruit matures, the soluble solids (sugars) content, pH, and aril color increases, while titratable acidity decreases. The cultivar "Wonderful" grown in California had an average soluble solids of 18.1%, 17% and titratable acidity value of 1.58%, 1.8%, when harvested in mid-October and late September, respectively (Kader et al., 1984; Elyatem & Kader, 1984). The maturity of the fruit is at a fully ripe state within 4 to 6 months after bloom, depending on weather conditions (Ben-Arie, Segal, Guelfat-Reich, 1984). Harvesting of the fruit should be done before they become overripe and crack open. Maturity index helps in selecting the fruit for harvesting which depends on the cultivar, and includes external skin color, juice color, acidity, and soluble solids content. For sweet cultivars, the maximum titratable acidity could be 1% and 1.5 - 2% for sweet-sour cultivars. The variation in the minimum soluble solids can vary from 15 - 17% (Kader et al., 1984; Elyatem et al., 1984; Ben-Arie et al., 1984). The flavor of the fruit is dependent on the sugar/acid ratio. They vary among different cultivars, and the best possible values for sweetness and astringency are generally a soluble solids level above 17% and total phenolics content below 0.25%. The cultivar "Wonderful" grown in California have minimum maturity indices of titratable acidity less than 1.85% and a red

color juice equivalent or darker than Munsel color chart 5R-5/12 (Kader et al., 1984; Elyatem et al., 1984).

Oxidative stress and generation of free radicals

The plants are exposed to various stress factors like drought, temperature, air pollution, light and limitation of nutrients. In response, the plants release reactive oxygen species and free radicals. When the balance between the oxidative and antioxidative capacity is disturbed, in favor of oxidants, it causes 'oxidative stress' (Sies, 1985). Reactive oxygen species (ROS) includes oxygen radicals and non-radical derivatives of oxygen, which can become radicals. The mitochondrial respiratory chain, microsomal cytochrome P450 enzymes, flavoprotein oxidases, and peroxisomal fatty acid metabolism are the major sources of ROS in eukaryotic cells. The common ROS includes superoxide, hydroxyl radical, hydrogen peroxide, and singlet oxygen (Devasagayam, Tilak, Boloor, Sane, Ghaskadbi, & Lele, 2004).

A free radical is defined as an atom or molecule that contains one or more unpaired electrons. They can be anionic, cationic or neutral, and the major free radical species that are studied extensively are those of oxygen (Bergendi, Beneš, Ďuračková, & Ferenčik, 1999). When the generation of free radicals is more, they can cause destructive and lethal cellular effects like apoptosis. The cellular respiration is shut down because they oxidize membrane lipids, cellular proteins, DNA, and enzymes (Antolovich, Prenzler, Patsalides, McDonald, & Rebards, 2001).

Free radicals lead to a number of diseases, like neurogenerative disorders (Alzheimer's disease), diabetes, and cardiovascular diseases (atherosclerosis). DNA is a major target of free radical damage, which result in mutations and give rise to cancer.

Accumulation of genetic changes leads to the development of cancer. Ageing is the result of mitochondrial ROS production and oxidative damage to mitochondrial DNA. Humans have endogenous defense mechanism by enzymes like superoxide dismutase, catalase, and glutathione peroxidase, along with vitamin E, uric acid and serum albumins. However, consumption of dietary antioxidants is also required (Antolovich et al., 2001).

Antioxidant

The antioxidant is defined as a substance in foods that when present at low concentrations compared to those of an oxidizable substrate significantly decreases or prevents the adverse effects of reactive species, such as reactive oxygen and nitrogen species (ROS/RNS), on normal physiological function in humans (Halliwell, Murcia, Chirico, & Aruoma, 1995; Huang, Ou, & Prior, 2005). Antioxidant activity and antioxidant capacity are often used, but they have different meanings. Roginsky & Lissi (2005) reported the "activity" describes the starting dynamics of antioxidant action and must be specified with reaction conditions like pressure and temperature. The antioxidant capacity gives the information about the duration of the antioxidant action. Various factors such as partitioning properties of the antioxidants between lipid and aqueous phases, oxidation conditions, and the physical state of the oxidizable substrate affect the antioxidant capacity in compound mixed foods and biological systems (Frankel & Meyer, 2000).

Antioxidants are classified as primary and secondary antioxidants. Dietary antioxidants capable of scavenging ROS/RNS to inhibit the radical chain reactions are known as primary chain-breaking antioxidants or free radical scavengers (FRS). When they are present in trace quantities, they delay or inhibit the initiation and propagation

steps by reacting with the peroxyl or alkoxyl radicals. Inhibiting the formation of the reactive oxidants are considered secondary or preventive antioxidants (Karadag, Ozcelik, & Saner, 2009). The efficiency of an antioxidant is dependent on the ability of the FRS to donate hydrogen to the free radical. Factors such as pH, volatility, sensitivity, and polarity affect the efficiency of phenolic FRS in foods (Karadag et al., 2009)

The preventive antioxidant enzymes include superoxide dismutase, catalase, and peroxidase. The various "preventive" antioxidation pathways include chelation of transition metals, singlet-oxygen deactivation, enzymatic ROS detoxification, UV filtration, inhibition of prooxidant enzymes, antioxidant enzyme cofactors, etc. (Laguerre, Lecomte, & Villeneuve, 2007). Decomposition of lipid peroxides and metal catalyzed initiation reactions are delayed as the metal chelators which are preventive antioxidants forms a complex with the transition metal ions. Frankel & Meyer (2000) listed the other antioxidant mechanisms such as singlet-oxygen quenching, oxygen scavenging, and blocking the prooxidant effects by binding specific proteins containing catalytic metal sites. The mechanisms like radical chain inhibitors, metal chelators, oxidative enzyme inhibitors, and antioxidant enzyme cofactors are often present in dietary antioxidants (Huang, Ou, & Prior, 2005).

A standardized method for measurement of antioxidant capacity is needed for appropriate comparison of different foods and commercial products, aiding in the correct application of assays, managing the deviations within or between samples, and providing standards for quality for regulatory purpose and for making health claims (Prior, Wu, & Schaich, 2005). The criteria for selection of any method for standardization must be based on its use over a long period of time in different laboratories. The other 'ideal'

necessities includes its relative simplicity, definite end point and chemical mechanism, use of biologically related radical source, measurement of the correct chemistry taking place in potential applications, easy availability of equipments, reproducibility of results, applicability for measurement of both hydrophilic and lipophilic antioxidants, and adaptability to "high-throughput" analysis for regular quality control analyses (Prior, Wu, & Schaich, 2005) .

Mechanisms of antioxidant action

The two major mechanisms by which antioxidants deactivate radicals are hydrogen atom transfer (HAT), and single electron transfer (SET). Regardless of the mechanism occurring, the final result of the reaction is the same, with the kinetics and potential of side reactions being different. The factors influencing the dominant mechanism in a system are the structure and properties of the antioxidant, solubility and partition coefficient, and solvent used in the system. The effectiveness of the antioxidant is dependent on bond disassociation energy (BDE) and ionization potential (IP) (Wright, Johnson, & DiLabio, 2001).

HAT methods measure the ability of an antioxidant to quench free radicals by hydrogen donation (AH = any H donor) (Prior et al., 2005)

$$X^{\circ} + AH \longrightarrow XH + A^{\circ}$$

These methods are related to the radical chain-breaking antioxidant capacity. BDE of the H-donating group of the potential antioxidant and IP determine the relative reactivity in HAT methods. These reactions are dependent on pH, solvent, and reach quick completion in seconds to minutes. Majority of the HAT-based methods observe the competitive reaction kinetics, and the kinetic curves help in quantification.

SET-based methods measure the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls, and radicals as shown in Fig. 2.4. (Wright et al., 2001). They are dependent on pH, and the relative reactivity is based on deprotonation and IP. With increase in pH, the IP values decrease demonstrating increased electron-donating capacity with deprotonation (Prior et al., 2005). The SET-based methods are dependent on the solvent as the charged species are stabilized by the solvent (Ou, Huang, Woodill-Hampsch, Flanagan, & Deemer, 2001). The reactions can be slow requiring more time for completion. They measure the relative percent decrease in product, instead of kinetics or total antioxidant capacity (Ozgen, Reese, Tulio, Scheerens, & Miller, 2006).

Methods to measure antioxidant capacity

The main features for any method include initiator of oxidation, suitable substrate, and measurement of end point. The initiators of oxidation may be increased temperature (Laguerre et al., 2007) and partial pressure of oxygen, addition of the transition metal catalysts (Ou et al., 2002), photosensitized oxidation by singlet oxygen by exposure to light (Min & Boff, 2002), and shaking to improve the contact between reactant and free radical sources (Pulido, Bravo, Saura-Calixto, 2000). However, different results may be obtained for the same food, due to the analytical methods used for measurement and the reaction conditions (Antolovich et al., 2002; Nilsson, Pillai, Onning, Persson, Nilsson, & Akesson, 2005).

Oxygen Radical Absorbance Capacity Assay (ORAC)

ORAC measures the inhibition of antioxidants of peroxyl-radical-induced oxidations by radical chain-breaking antioxidant activity by H-atom transfer (Ou,

Woodill-Hampsch, & Prior, 2001). Thermal decomposition of ABAP (2,2'-azobis(2-amidinopropane) dihydrochloride) in aqueous buffer gives peroxyl radicals, while hydroxyl radicals are produced from Cu^{2+}-H_2O_2 (Cao, Sofic, & Prior, 1997). The radicals react with an oxidizable protein substrate, which is a fluorescent probe and then becomes a non fluorescent product. The loss in fluorescence is measured over a period of time for quantification. Previously, B-phycoerythrin, a fluorescent protein was used as the probe, mainly because of its high fluorescent yield, excitation wavelengths, sensitivity to ROS, and water solubility. The standard used is Trolox, which is diluted in four to five different concentrations for constructing the standard curve. The samples, control, and standard are mixed with fluorescein solution and incubated at a constant temperature of 37 °C. Then ABAP is added to initiate the reaction (MacDonald-Wicks, Wood, & Garg, 2006). 1 mol of ABAP looses a dinitrogen to produce 2 mol of ABAP radical. The ABAP radical reacts with oxygen to produce a stable peroxyl radical ROO°**.** The loss of fluorescence of the probe indicates the extent of damage caused by its reaction with the peroxyl radical. The intensity of fluorescence with excitation at 485 nm and emission at 525 nm is measured for every minute at pH 7.4 and 37 °C. Decay of fluorescence is prevented when an antioxidant is present (Ou et al., 2002).

Protective effects of an antioxidant is measured by the net area under the fluorescence decay curve ($AUC_{sample} - AUC_{blank}$) (Fig. 2.5), and the single value accounts for lag time, initial time, and total inhibition (Prior et al., 2005). The other advantages include use of fully automated microplate fluorescence reader which is readily accessible with high efficiency, and inexpensive fluorescent probe (Huang, Ou, Hampsch-Woodill, Flanagan, & Deemer, 2002). The reaction is highly temperature sensitive and incubation

of the reaction buffer at 37 °C before addition of ABAP decreased the intra-assay variability (Prior, Hoang, Gu, Wu, Bacchiocca, Howard et al., 2003).

Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay is completely based on electron transfer mechanism, where the ability of phenolics to reduce yellow ferric tripyridyltriazine complex (Fe(III)-TPTZ) to blue ferrous complex (Fe(II)-TPTZ) is measured using a spectrophotometer at 593 nm (Benzie & Strain, 1999) (Fig. 2.6). The measured value is linearly related to the total reducing capacity of electron-donating antioxidants. FRAP reactions are carried out at acidic pH of 3.6 to maintain the solubility of iron. The reduction of 1 mol of Fe (III) to Fe (II) is defined as one FRAP unit (Huang et al., 2005).

The FRAP assay is simple, rapid, inexpensive, robust, and does not need any special equipments. The disadvantages include its inability to measure compounds which act by radical quenching (H transfer). Pulido et al. (2000) reported that the absorption at 593 nm for polyphenols like caffeic, ferulic, ascorbic, and quercetin does not end at 4 min, but it increases even after few hours after reaction time. Therefore, the FRAP values obtained by using a fixed end point may not represent a completed reaction.

Trolox Equivalent Antioxidant Capacity (TEAC)

Miller, Rice-Evans, Davies, Gopinathan, & Milner (1993), first reported TEAC assay, based on the scavenging ability of antioxidants to the long-life radical cation $ABTS^{\circ+}$ (Fig. 2.7). The intensely colored radical cation can be monitored spectrophotometrically in the range of 415 - 815 nm, and is produced by oxidation of ABTS by peroxyl radicals. The wavelengths of 415 and 734 nm were used extensively by many investigators. The antioxidant capacity is measured as the ability of the test

compounds to decrease the color when reacting with the $ABTS^{\circ+}$ radical, at a fixed time point (4 - 6 min). The results of the test compounds are reported relative to Trolox (Roginsky et al., 2005). The modified methods generates the free radical by chemical reactions (manganese dioxide, potassium persulfate, ABAP), and enzymatic (peroxidase, myoglobin) reactions.

The assay is relatively simple where $ABTS^{\circ+}$ radical reacts rapidly with antioxidants, and can be used over a wide pH range (Ozgen et al., 2006). However, generation of the radical by chemical reactions takes up to 16 h. It can be used to determine both hydrophilic and lipophilic antioxidant capacities, and can also be adapted to microplates (Erel, 2004; Chen, Chang, Yang, & Chen, 2004). The ABTS radical used is a "nonphysiological" radical source as it is not found in our body. The end point of 6 min may not be suited for slow reactions, which may take a longer time to reach completion (Prior et al., 2005).

Folin-Ciocalteu (F-C) or Total Phenolics method

The Folin-Ciocalteu reagent (FCR) has phosphomolybdic/phosphotungstic acid complexes which react with the electrons from the phenolic compounds in an alkaline medium to yield molybdenum, a blue colored product (Fig. 2.8). This can be monitored spectrophotometrically at 750-765 nm (Folin, 1927). The improved method was developed by Singleton & Rossi (1965). To minimize the variability and prevent inconsistent results, (1) proper volume ratio of alkali and FCR, (2) optimal reaction time and temperature for color development, (3) monitoring of optical density at 765 nm, minimizing interference from sample matrix, and (4) use of reference standards like gallic acid should be used (Prior et al., 2005).

The total phenolic assay is very simple, convenient, and reproducible, thus widely used in a number of laboratories (Huang et al., 2005). Good correlations existed between the total phenolic assay by FCR and antioxidant capacity methods (FRAP, TEAC, ORAC, etc.) (De Beer, Joubert, Gelderbloom, & Manley, 2003; Shahidi, Liyana-Pathirana, & Wall, 2006; Stratil, Klejdus, & Kuban, 2006). The choice of standard is critical as the absorbance values are proportional to the number of reacting phenolic hydroxyl groups, and also the molecular structure. The FCR reagent is non-specific and it can also be reduced by other non-phenolic compounds (MacDonald-Wicks et al., 2006).

Taste of the fruit

The taste of the fruit is generally determined by the organic acid: sugar ratio.

Organic acids

The distribution of organic acids is widespread in various fruits and vegetables. With development of the fruit, the accumulation of organic acid increases. They are used as respiratory substrates during ripening of the fruit. The two major acids found in fruits, malic and citric acid are synthesized in different parts of the fruit cells. Malic acid was synthesized in the cytosol by phosphoenolpyruvate carboxylase and NAD-dependent malate dehydrogenase. Citric acid accumulation was carried out by mitochondrial citrate synthesis (Diakou, Svanella, Gaudillere, & Moing, 2000). The organic acids act as food acidulants and also aid in determination of authenticity of juices. The most common and widely used acid is citric acid. Malic and tartaric acid are found in fruits and are used in fruit flavored drinks. The color of the juice is related to the quantity of organic acids present. Fruit juices have a low pH, because of the acidity contributed by presence of organic acids. This has an effect on the shelf life as it inhibits the growth of

microorganisms. Organic acids also influence the flavor, stability, acceptability, and keeping quality of the juice. Therefore, determination of individual organic acids is essential for quality control and labeling purposes (Shui & Leong, 2002).

Sugars

The determination of sugars is important for the food industry. The organoleptic quality of the juice depends on the level of sugars present. It also has an influence on other characteristics such as flavor, maturity, quality, and authenticity of juice. The total soluble sugars (TSS) increase during maturation and ripening (Shwartz, Glazer, Bar-Ya'akov, Matityahu, Bar-Ilan et al., 2009). Determination of individual sugars is performed using high performance liquid chromatography (HPLC). The main types of chromatography used are reversed phase with bonded phases, ion exchange, and ion exclusion. The mobile phases which are commonly used for the HPLC separation of sugars are mixtures of water/acetonitrile, NaOH solutions, HPLC-grade water, sulfuric acid solutions or gradient elution systems. Traditionally, refractive index (RI) detector was used for detection of sugars. Other types of detectors used are evaporative light-scattering detector (ELSD), photodiode array detector (PDA), electrochemical detection, and Fourier transform infrared spectroscopy (FTIR). The ELSD is a suitable detector in the determination of sugars, because the response is almost similar for all non-volatile solutes. It also provides good sensitivity and a stable chromatographic baseline, compared to the RI detectors (Martínez Montero, Rodríguez Dodero, Guillén Sánchez, & Barroso, 2004).

Phenolic compounds

The secondary metabolites synthesized in the plant during normal development and under stressful conditions like infection, wounding, and UV radiation are called phenolic compounds (Harborne, 1982; Beckman, 2000; Shahidi & Naczk, 2004). All the phenolic compounds have a basic, common structural unit, the phenol. It is an aromatic ring having atleast one hydroxyl substituent. The number and position of hydroxyl groups on the aromatic ring is different for the various classes of phenols. The plant phenolics comprise of simple phenols, phenolic acids (benzoic and cinnamic acid derivatives), coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans, and lignins (Table 2.1) (Croteau, Kutchan, & Lewis, 2000; Shahidi et al., 2004).

The functions of plant phenols include primary metabolism, growth, protection of the cell components against photooxidation by ultraviolet light, and disease resistance (Parras Rosa, 1996). Plant phenolics possess antioxidant activity, and influence the physiological activity. Other functions include its ability to scavenge active oxygen species and electrophiles, ability to inhibit nitrosation, to chelate metal ions, potential for autooxidation and ability to alter some cellular enzyme activities (Cuvelier, Berset, & Richard, 1994; Dziedzic, & Hudson, 1984; Houlihan, Ho, & Chang, 1984; Onyeneho & Hettiarachchy, 1992).

Phenolic acids

Phenolic acids contain one carboxylic acid functionality. In plant metabolites, they refer to a distinct group of organic acids. These phenolic acids occur naturally and contain two distinctive carbon frameworks: hydrocinnamic and hydrobenzoic structures (Table 2.2). The fundamental structure stays the same with changes only in the number and position of hydroxyl groups on the aromatic ring resulting in various phenolic acids.

Caffeic, *p*-coumaric, vanillic, ferulic, and protocatechic are acids found in almost all the plants. Other acids like gentisic, syringic are only found in selected foods or plants (Shahidi & Wanasundara, 1992).

The biosynthetic origin of benzoic and cinnamic acid derivatives is from the aromatic amino acid L-phenylalanine. It occurs in three steps and is called the "general phenylpropanoid metabolism." The amino acid L-phenylalanine is synthesized from chorismate, which is the final product of shikimate pathway (Herrmann, 1995). The soluble phenolics are present in the cell walls and the insoluble phenolics are found in the plant cell vacuoles (Stalikas, 2007). The major fraction of the acids are linked through ester, ether, or acetal bonds to cellulose, proteins, flavonoids, glucose, and terpenes (Klick & Herrmann, 1988; Winter & Herrmann, 1986). Free acids forms are found in very small fractions. The growing conditions like temperature are known to affect the phenolic acid content (Zheng & Wang, 2001).

Phenolic acids play an important role in food quality. They have been known to influence color, sensory qualities, nutritional, antioxidant, and organoleptic (flavor, astringency, and hardness) properties of foods (Tomás-Barberán & Espín, 2001; Maga, 1978; Peleg, Naim, Rouseff, & Zehavi, 1991). The content and phenolic acid profile would help in understanding the effect on fruit maturation, prevention of enzymatic browning, and their use as food preservatives (Tomás-Barberán et al., 2001). The antioxidant activity of phenolics is due to the reactivity of the phenol moiety (hydroxyl substituent on the aromatic ring). The major mechanism of antioxidant activity is by radical scavenging through hydrogen atom donation. The radical-quenching ability

depends on the substituents on the aromatic ring, and thus different acids have different antioxidant activity (Shahidi et al., 1992; Rice-Evans, Miller, & Paganga, 1996).

The role of phenolic acids as dietary antioxidants is one of the prominent health benefits that has gained increased attention in recent years. They are found abundantly in plant-based foods, and their estimated range for human consumption is 25 mg - 1 g a day based on diet (fruit, vegetables, grains, teas, coffees, spices) (Clifford, 1999). Other biological activities of phenolic acids, specifically caffeic acid, includes selective blocking of the biosynthesis of leukotrienes, components involved in immunoregulation diseases, asthma, and allergic reactions (Koshihara, Neichi, Murota, Lao, Fujimoto, & Tatsuno, 1984).

Flavonoids

These phenolic compounds have atleast two phenol subunits, and they are found in almost all the plants. They are formed from the aromatic amino acids, phenylalanine, tyrosine, and malonate (Harborne, 1986). The basic structure includes the flavan nucleus, which has 15 carbon atoms arranged in three rings (C6-C3-C6), labeled A, B, and C (Fig. 2.9). The variation in structure arises from the degree and pattern of hydroxylation, methoxylation, prenylation, or glycosylation. If the flavonoids structure has three or more phenol subunits, they are called tannins (hydrolysable and non-hydrolysable). Different classes of flavonoids include flavones, flavanones, isoflavones, flavonols, flavanonols, flavan-3-ols, anthocyanidins, and anthocyanins (Stalikas, 2007).

Flavonoids are one of the most commonly found pigments, after chlorophyll and carotenoids. Their physiological roles are varied and they are found in plants as glycosylated derivatives. The attractive colors of flavones, flavonols, and anthocyanidins

may act as visual signals for pollinating insects. Catechins and other flavonols are astringent which might serve as a defence system against insects harmful to plants (Mazza & Miniati, 1993). Catalytic functions in the light phase of photosynthesis and/or regulators of ion channels involved in phosphorylation are observed. The ROS produced in the plant cells during photosynthetic electron transport system are scavenged by flavonoids, thus acting as stress protectants. The UV-absorbing property of flavonoids also aids in preventing the plants against UV radiation of the sun and scavenge the ROS generated by them. They also contribute significantly to the human diet and their intake is in the range of 50 to 800 mg a day (Stalikas, 2007).

Anthocyanins

They are the glycosylated derivatives of 3,5,7,3'-tetrahydroxyflavylium cation (Fig. 2.10). The occurrence of glycosylation occurs at the 3,5, and 7 positions. Anthocyanidins are the non-glycosylated molecule (aglycone). The anthocyanins contain sugars and acylated sugars. The common sugars which are monosaccharides include glucose, galactose, arabinose and rhanmose. The acyl substituents are *p*-coumaric, caffeic, ferulic or sinapic acids which are generally bonded to the C-3 sugar (Lee, 1992).

The red, blue, and purple colors of different fruits and vegetables are due to the presence of anthocyanins. They are quite unstable during processing and storage leading to its degradation. The total level of anthocyanin pigments are measured to help in assessing the quality of color in different foods. They are also potential sources of safe food colorants in the food industry. The anthocyanins may also possess various health benefits such as reduction of coronary heart disease (Bridle & Timberlake, 1996), increased visual acuity (Timberlake & Henry, 1988), antioxidant and anticancer

properties (Wang, Cao, & Prior, 1997; Kamei, Kojima, Hasegawa, Koide, Umeda, Yukawa et al., 1995). A fast and easy way to analyze total monomeric anthocyanin levels relies on the structural transformation of the anthocyanin chromophore as a function of pH. They permit reliable measurement of total anthocyanins even in the presence of polymerized degraded pigments and other interfering compounds. This helps in determining the quality of anthocyanin-containing food products (Lee, 1992). During maturation of different fruits, significant changes in the accumulation of anthocyanins occur. Therefore, quantitative determination of individual anthocyanins by RP-HPLC provides a good understanding of their development during maturing of different fruits like red tart cherry (Dekazos, 1970), thornless blackberry (Sapers, Hicks, Burgher, Hargrave, Sondey, & Bilyk, 1986), and red grape (Fernández-López, Hidalgo, Almela, & López-Roca, 1992). The unique anthocyanin fingerprint has been used to verify the authenticity of fruit juices and its products which are rich in anthocyanins.

Tannins

They can be defined as a unique group of phenolic metabolites of relatively high molecular weight in the range of 3000 to 30000, having the ability to complex strongly with carbohydrates and proteins (Porter, 1989). Tannins can be classified into three groups namely, condensed tannins, hydrolysable tannins, and complex tannins as shown in Fig. 2.11 (Khanbabaee & Van Ree, 2001).

Several reports suggest that the intake of tannins may delay the occurrence of chronic diseases. The biological effects of tannins may be exerted in two ways: 1) as a non-absorbable, complex structure with binding properties which may produce local effects in the gastrointestinal tract (antioxidant, radical scavenging, antimicrobial,

antiviral, antimutagenic and antinurient effects), or 2) as absorbable tannins (low molecular weight) and absorbable metabolites from colonic fermentation of tannins that may produce systemic effects in different organs. Other ways by which the tannins may act are by complexation with metal ions, antioxidant and radical scavenging activities or their ability to complex with molecules like proteins and polysaccharides (Haslam, 1996).

Condensed tannins

They are also called proanthocyanidins. They are oligomeric or polymeric flavonoids containing flavan-3-ol (catechin) units. Polymerization is a result of action of enzymes or acids. The ability to precipitate proteins depends on the degree of polymerization. High levels of condensed tannins during wine making can produce a dry feeling inside your mouth (Vermerris & Nicholson, 2007). The major sources of proanthocyanidins in the diet are fruits including berries, wine, beer, and other commonly consumed fruit juices. In the United States, the mean intake of proanthocyanidins with a degree of polymerization greater than 2 is 53.6 mg/day/person (Serrano, Puupponen-Pimia, Dauer, Aura, & Saura-Calixto, 2009).

Hydrolysable tannins

Polyesters formed between a sugar moiety (or other non-aromatic polyhydroxy compounds) and organic acids results in hydrolysable tannins. These compounds undergo hydrolytic cleavage in the presence of diluted acids to produce respective sugar and acid moiety. Primarily, the sugar moiety is glucose, but fructose, xylose, and saccharose are also found. If the organic acid present is gallic acid, then they form gallotannins. Ellagitannins are esters with hexahydroxydiphenic acid. They form ellagic acid when hydrolyzed through the elimination of water.

Gallotannins are not widely distributed and are found in woody and herbaceous plants. Ellagitannins are found in almost all the berries and their products such as jams, jellies, juices, pecans, walnuts, peanuts, blue plum, pomegranate (fruit and juice), red apple, white and red grapes (Serrano et al., 2009). It was reported by Seeram, Lee, Hardy & Heber (2005) that the total level of native ellagitannin and other sources of ellagic acid in pomegranate juice was 1770 mg/L, with punicalagin being the main ellagic acid.

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–Different parts of the pomegranate fruit (A). B: pomegranate juice; C: section of pomegranate; D: pomegranate peel; E: pomegranate arils; F: pomegranate seeds.

Figure 2.1 Different parts of the pomegranate fruit (Viuda-Martos et al., 2010)

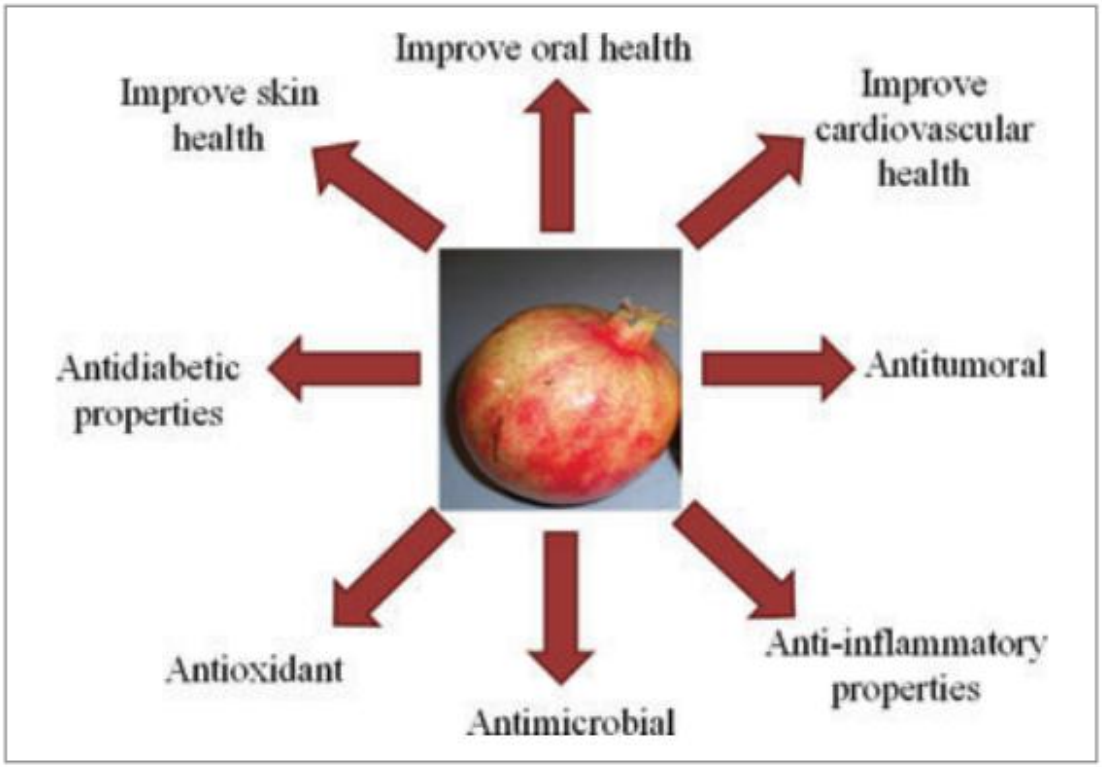


Figure 2.2 Primary functional and medicinal effects of pomegranate (Viuda-Martos et al., 2010)

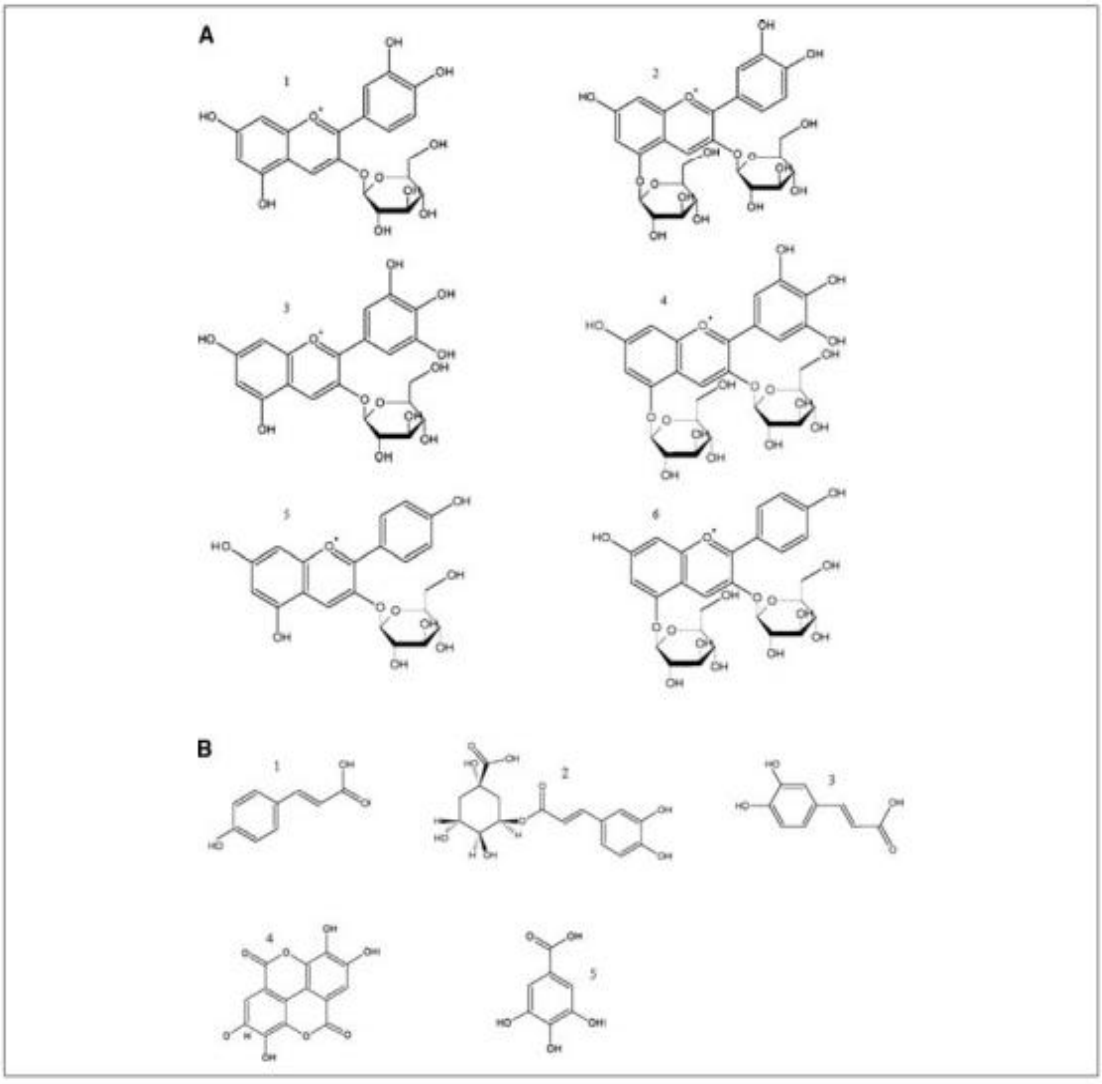


Figure 2.3 A) Principal anthocyanins present in pomegranate juice. 1: cyanidin-3-O-glucoside; 2: cyanidin-3,5-di-O-glucoside; 3: delphinidin-3-O-glucoside; 4: delphinidin-3,5-di-O-glucoside; 5: pelargonidin-3-O-glucoside; 6: pelargonidin-3,5-di-O-glucoside. (B) Principal phenolic acids present in pomegranate juice: 1: *p*-coumaric acid; 2: chlorogenic acid; 3: caffeic acid; 4: EA; 5: gallic acid.

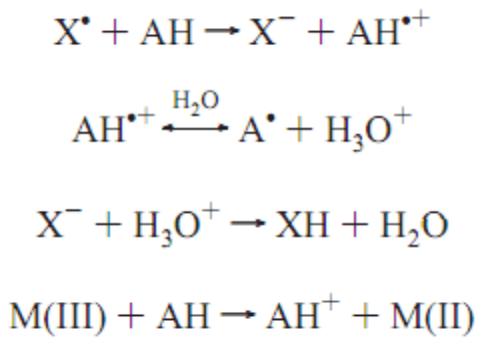


Figure 2.4 SET-based mechanism (Wright et al., 2001)

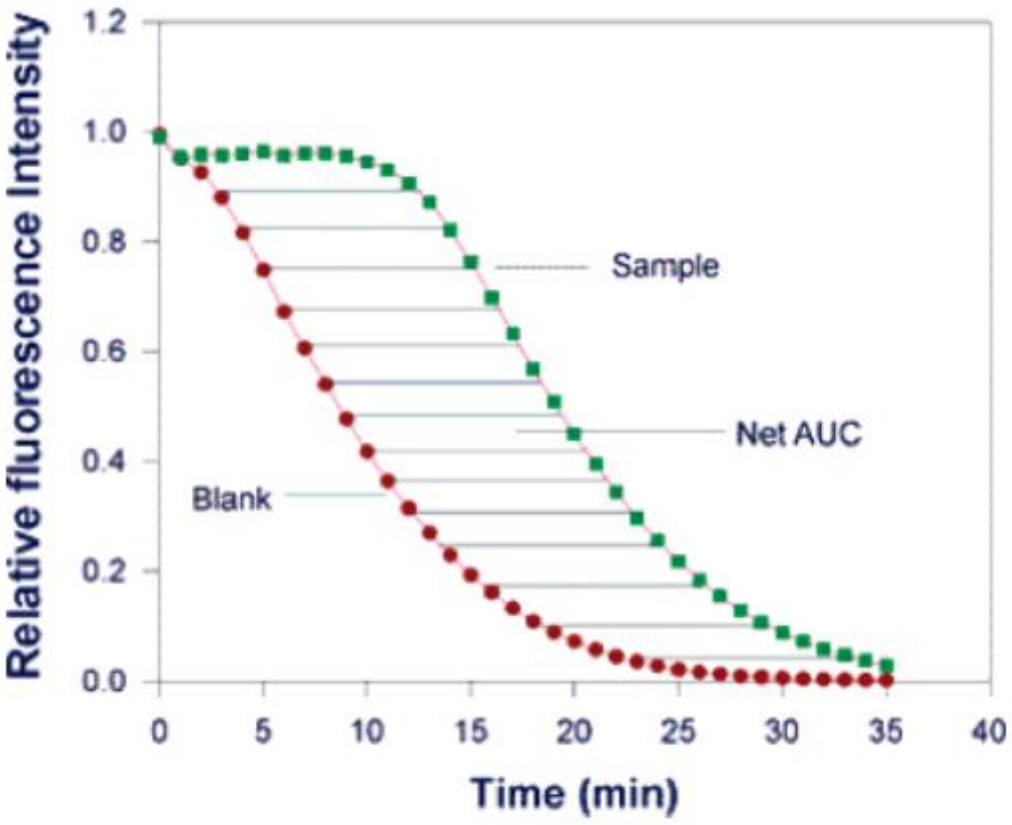


Figure 2.5 ORAC antioxidant activity expressed as net area under the curve (AUC) (Prior et al., 2005).

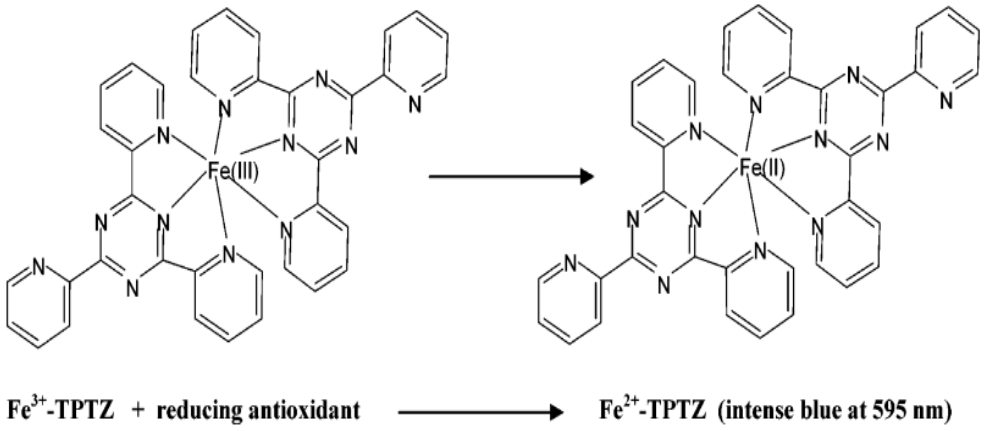


Figure 2.6 FRAP reaction (Prior et al., 2005)

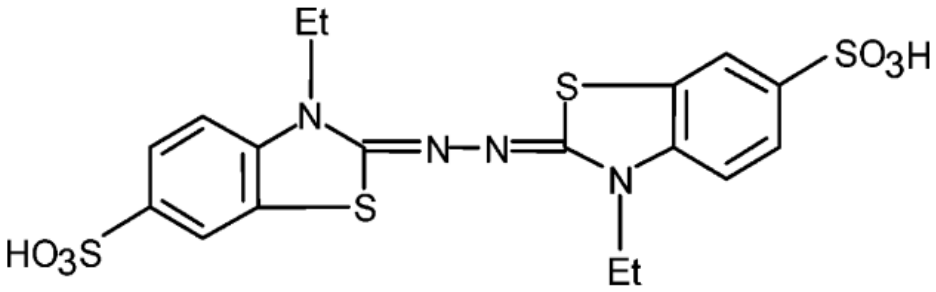


Figure 2.7 Structure of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\circ+}$) (Prior et al., 2005)

$$Na_2WO_4/Na_2MoO_4 \rightarrow (phenol-MoW_{11}O_{40})^{-4}$$

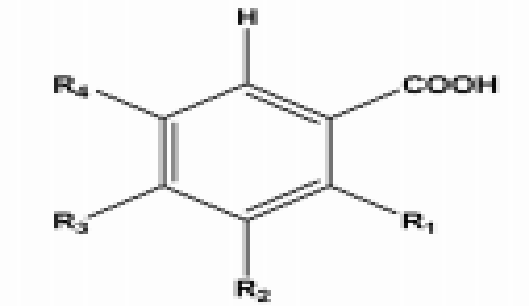
$$Mo(VI)\ (yellow) + e^{-} \rightarrow Mo(V)\ (blue)$$

Figure 2.8 Total phenolic reaction using Folin-Ciocalteu reagent (Folin, 1927)

Table 2.1 Different classes of phenolic compounds (Harborne et al., 1964)

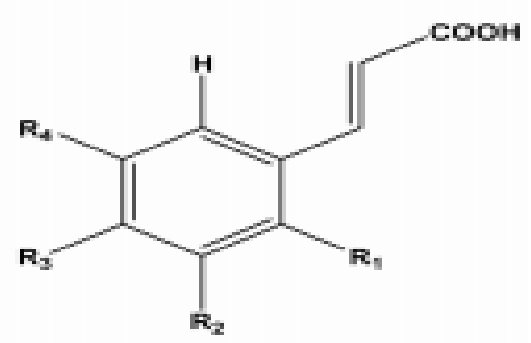
Structure	Class
C_6	simple phenolics
C_6 - C_1	phenolic acids and related compounds
C_6 - C_2	acetophenones and phenylacetic acids
C_6 - C_3	cinnamic acids, cinnamyl aldehydes, cinnamyl alcohols
C_6 - C_3	coumarins, isocoumarins, and chromones
C_{15}	chalcones, aurones, dihydrochalcones
C_{15}	flavans
C_{15}	flavones
C_{15}	flavanones
C_{15}	flavanonols
C_{15}	anthocyanidins
C_{15}	anthocyanins
C_{30}	biflavonyls
C_6-C_1-C_6, C_6-C_2-C_6	benzophenones, xanthones, stilbenes
C_6, C_{10}, C_{14}	quinones
C_{18}	betacyanins
Lignans, neolignans	dimers or oligomers
Lignin	polymers
Tannins	oligomers or polymers
Phlobaphenes	polymers

Table 2.2 Structure of the major phenolic acids in nature (Stalikas, 2007)



Hydroxybenzoic Acids

Name	R_1	R_2	R_3	R_4
Benzoic acid	H	H	H	H
p-Hydroxybenzoic acid	H	H	OH	H
Vanillic acid	H	OCH_3	OH	H
Gallic acid	H	OH	OH	OH
Protocatechuic acid	H	OH	OH	H
Syringic acid	H	OCH_3	OH	OCH_3
Gentisic acid	OH	H	H	OH
Veratric acid	H	OCH_3	OCH_3	H
Salicylic acid	OH	H	H	H



Hydroxycinnamic Acids

Name	R_1	R_2	R_3	R_4
Cinnamic acid	H	H	H	H
o-Coumaric acid	OH	H	H	H
m-Coumaric acid	H	OH	H	H
p-Coumaric acid	H	H	OH	H
Ferulic acid	H	OCH_3	OH	H
Sinapic acid	H	OCH_3	OH	OCH_3
Caffeic acid	H	OH	OH	H

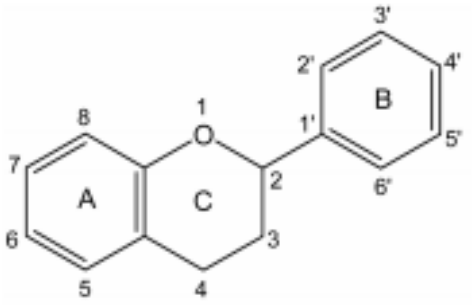
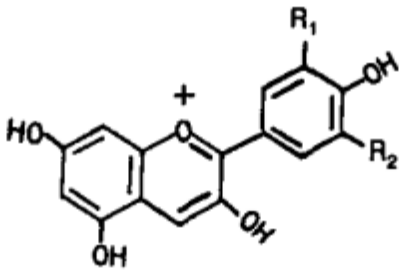


Figure 2.9 Basic structure of a flavonoid (Stalikas, 2007)



	R_1	R_2
Delphinidin	OH	OH
Cyanidin	OH	H
Petunidin	OCH_3	OH
Pelagonidin	H	H
Peonidin	OCH_3	H
Malvidin	OCH_3	OCH_3

Figure 2.10 Structural formulas of the different anthocyanins (Lee et al., 1992)

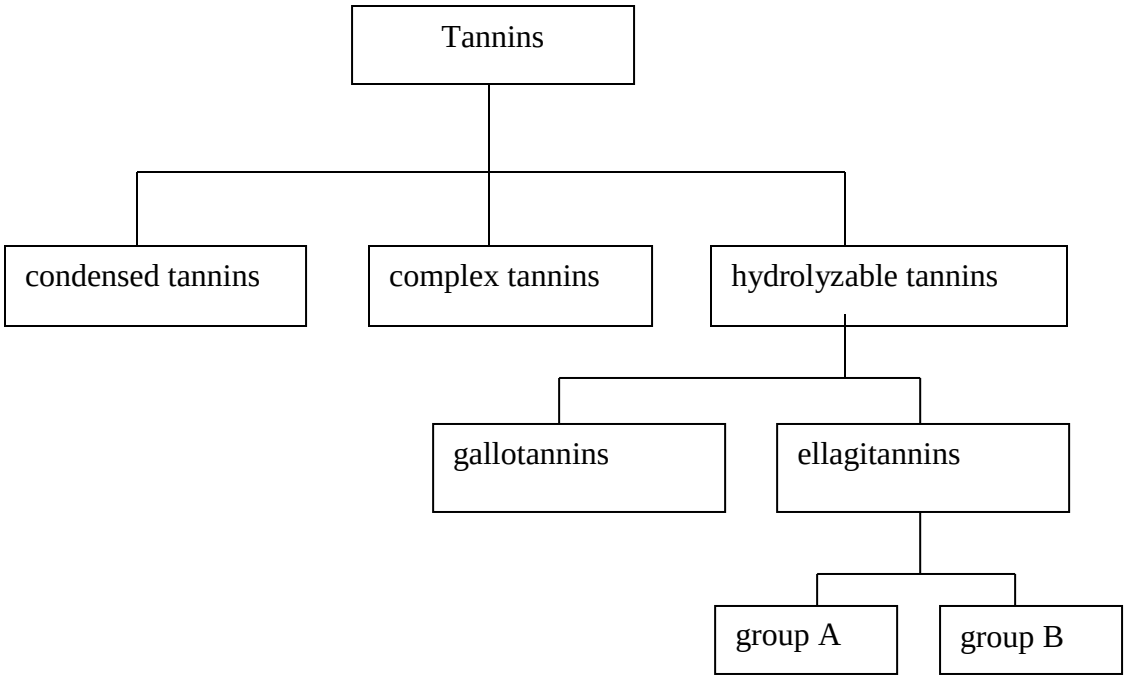


Figure 2.11 Classification of tannins (Vermerris et al., 2006)

CHAPTER 3

CHARACTERIZATION OF ARIL JUICE OF GEORGIA-GROWN POMEGRANATE CULTIVARS EXTRACTED BY TWO DIFFERENT METHODS

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Abstract

Pomegranate juice is well recognized for its phytonutrient content. The objective of this study was to evaluate and quantify the effect of blender and mechanical press extraction methods on juice yield and antioxidant properties of fourteen pomegranate cultivars grown in Georgia. Folin-Ciocalteau method was used to determine the total polyphenols. Antioxidant capacity was studied using ferric reducing antioxidant power (FRAP), Trolox equivalent antioxidant capacity (TEAC), and oxygen radical absorbance capacity (ORAC) assays. The juice yield averaged 30.61% of fresh weight (FW) of the fruit for blender and 24.56% for mechanical press. Total polyphenols and total monomeric anthocyanins were higher in blender (57.41 mg gallic acid equivalents (GAE)/100 g FW; 12.01 mg cyanidin 3-glucoside equivalents/100 g FW) compared to mechanical press (45.00 mg GAE/100 g FW; 9.53 mg cyanidin 3-glucoside equivalents/100 g FW), respectively. The organic acids, sugars and phenolic compounds were quantified using HPLC. Significant differences in the chemical properties of the aril juice were found after extraction by the two methods.

Keywords: Extraction methods; yield; antioxidant capacity; polyphenols; organic acids; *Punica granatum* L.

Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest known fruits which has gained increased attention in recent years due to its tremendous health benefits (Tezcan, Gültekin-Özgüven, Diken, Özçelik, & Erim, 2009). Several research studies have shown that the fruit contain certain anticarcinogenic (Bell & Hawthorne, 2008), antimicrobial (Reddy, Gupta, Jacob, Khan, & Ferreira, 2007), and antiviral compounds (Kotwal, 2007). Epidemiological studies conducted within the last few years have confirmed that certain compounds in pomegranate juice can decrease the oxidation of low-density lipoproteins (LDL), significantly reduce blood pressure and have antiatherosclerotic effects (Gil, Hess-Pierce, Holcroft, & Kader, 2000).

Pomegranate juice has a high antioxidant capacity, approximately three times greater than those of red wine and green tea (Gil et al., 2000). The antioxidative properties of pomegranate polyphenols (catechins, ellagic tannins, gallic and ellagic acids), sugar-containing polyphenolic tannins and anthocyanins (cyanidin 3-glucoside, cyanidin 3,5-diglucoside and delphinidin 3-glucoside) are responsible for the health effects provided by pomegranate juice (Gil et al., 2000; Aviram, Dornfeld, Rosenblat, Volkova, Kaplan, & Coleman, 2000). The increased public awareness about the importance of functional foods and the health benefits of pomegranates has given rise to a greater demand in the Western world for pomegranates and its products. This trend gave rise to the extensive pomegranate grown in different regions of the world, and development of industries that produce pomegranate products (Holland, Hatib, & Bar-Ya'akov, 2008). The edible part of the pomegranate fruit termed the arils, can be yellow to deep red in color. They consist of around 80% juice and 20% seeds by weight (Özgen,

Durgaç, Serçe, & Kaya, 2008). The increased market demand has led to characterization of the different varieties to obtain a superior quality product with economical significance (Martínez, Melgarejo, Hernàndez, Salazar, & Martínez, 2006).

Pomegranate juice composition is dependent on the processing method used which significantly affects the chemical properties of the juice. Tzulker, Glazer, Bar-Ilan, Holland, Aviram & Amir (2007) reported that the juice obtained from arils alone has poor antioxidant capacity and polyphenol content in comparison to the juice obtained from the whole fruit using a juice extractor. Miguel, Dandlen, Antunes, Neves & Martins (2004) showed that there were no significant differences in the level of sugars and organic acids in juices obtained by seed centrifugation and electric squeezer. Similar findings were reported by others (Dafny-Yalin, Glazer, Bar-Ilan, Kerem, Holland, & Amir, 2010).

The objective of this study was to evaluate and compare the juice yield potential and the chemical characteristics of fourteen Georgia-grown pomegranate cultivars using two different juice extraction methods.

Materials and methods

Plant material

Fourteen pomegranate (*P. granatum*, Punicaceae) cultivars grown in Georgia were used in this study. White Don Wade and Turk Don Wade were harvested from a grower located near Alma, GA, while the remaining cultivars (Haku-botan, Don Sumner South Tree, Don Sumner North Tree, Mejhos, Salavatski, Kaj-acik-anor, Nikitski ranni, Afganski, Entek Habi Saveh, Eve, Cranberry, and Cloud) were obtained from the University of Georgia Ponder farm, located near Tifton, GA. The trees at the Ponder

Farm were planted in a loamy-sand soil (sand, 86%; silt, 7%; and clay, 7%) from 1990 to 1993. Orchard management was minimal until 2008, with no supplemental fertilizer or irrigation applied. Pruning was performed at irregular intervals since the initial planting. Fruits were harvested at maturity, as estimated based on soluble sugar content, color, and total acidity, then transported to the University of Georgia Vidalia Onion Research Laboratory, where fruits were cooled to 7 °C prior to subsequent analysis.

Chemicals

Pure standards of succinic acid, DL-malic acid, oxalic acid, gallic acid, (+)- catechin, (-)- epicatechin, caffeic acid, *p*-coumaric acid, ferulic acid, ellagic acid, quercetin, punicalagin, Folin-Ciocalteu reagent, 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), citric acid, and potassium persulfate were purchased from Sigma Chemical Co. (St. Louis, MO). 2, 4, 6-Tripyridyl-s-triazine (TPTZ) and 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Acros Organics (Morris Plains, NJ). L-Ascorbic acid was from Mallinckrodt Baker Inc. (Phillipsburg, NJ) and $FeCl_3.6H_2O$) from Fluka (Milwaukee, WI). Other solvents and chemicals were purchased from Sigma Chemical Co., J. T. Baker Chemical Co. (Phillipsburg, NJ), and/ or Fischer Scientific (Norcross, GA).

Sample preparation

The fruits were washed with water and wiped completely dry. Fruits from each cultivar were then divided into equal portions for juice extraction with either an Oster® blender (Oster, Fort Lauderdale, FL) or hand operated juice extractor/mechanical press (Strite-Anderson Mfg. Co., Minneapolis, MN). The juice was obtained by pressurization of the arils. In the blender, the white membrane and the arils were juiced while in the

juice extractor, it was only the aril juice (Fig. 3.1a) All sample preparation was done under dark conditions. The juice was flushed with nitrogen and stored at –80 °C until further analysis. All extractions were performed in triplicate.

Dry Weight (DW) Determination

DW was determined according to the guidelines of AOAC (1990). Sample dry weight [g/g of fresh weight (FW)] was calculated as shown below.

$$DW = (c - a) / (b - a)$$

where *a* is the weight of the empty pan (g), *b* is the weight of the pan and fresh sample (g), and *c* is the weight of the pan and dried sample (g). All samples were analyzed in triplicate, and average values were reported.

Total polyphenols (TPP)

Total polyphenols were determined according to the Folin-Ciocalteu reagent method (Singleton & Rossi, 1965). To each 50 μL of extracted juice sample, 0.5 mL of Folin-Ciocalteu reagent and 1.5 mL of 7.5% sodium carbonate solution were added. The samples were then mixed well and allowed to stand for 30 min in the dark at room temperature. Absorption at 765 nm was read using a Shimadzu 300 UV-vis spectrophotometer (Shimadzu UV-1601, Norcross, GA). Quantification was based on the standard curve generated with 1-15 mg/L of gallic acid, and average results from triplicate determinations are reported as mg of GAE/100 g of FW.

Total anthocyanins

The total anthocyanin content was estimated by the pH-differential (AOAC method 2005.02) using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M) on a UV-vis spectrophotometer (Shimadzu UV-

1601, Norcross, GA). Samples were diluted in pH 1.0 and pH 4.5 buffers and then measured at 520 and 700 nm. The absorbance was calculated as $A = (A_{520nm} - A_{700nm})_{pH\ 1.0} - (A_{520nm} - A_{700nm})_{pH\ 4.5.}$

The monomeric anthocyanin pigment concentration was calculated as cyanidin-3-glucoside. The monomeric anthocyanin pigment (mg/L) = A x MW x DF x 1000/(ε x 1), where A = absorbance, MW = molecular weight (449.2), DF = dilution factor, and ε = molar absorptivity (26900). All measurements were done in triplicate and averages were reported.

Antioxidant capacity

Ferric reducing antioxidant capacity (FRAP) assay

The FRAP assay was performed according to the method of Benzie & Strain (1996) with minor modifications. Stock solutions of 300 mM acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine solution in 40 mM HCl), and 20 mM $FeCl_3.6H_2O$ were prepared. The FRAP reagent was prepared by mixing the stock solutions in 10:1:1 ratio and maintained at 37 °C and pH 3.6. Then, 10 µL of the sample and 300 µL of FRAP reagent were added in a 96-well microplate (Tsao, Yang, Xie, Sockovie, & Khanizadeh, 2005) and incubated at room temperature for 4 min. The absorbance was measured at 595 nm using a microplate reader (BioRad 680 XR, Hercules, CA). Trolox calibration solutions (100, 200, 400, 500 and 750 µM) were used to generate the standard curve and the results were expressed as micromoles of Trolox equivalents (TE)/g of FW. All assays were done in triplicate and averages were reported.

Trolox equivalent antioxidant capacity (TEAC) assay

The assay was performed based on the method of Lee, Kim, Kim, Lee, & Lee (2003) with slight modifications. Briefly 7 mM ABTS solution and 2.45 mM potassium persulfate solution were mixed and kept in the dark at room temperature for 12-16 h. The $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. To each 10 µL aliquot of Trolox standard or sample, 200 µL of diluted $ABTS^{\cdot+}$ was added , and the absorbance was read for 6 min at 734 nm using a microplate reader (BioRad 680 XR, Hercules, CA). The percent inhibition of absorbance was calculated and plotted as a function of Trolox concentration. TEAC values of samples were calculated from the standard curve and reported as micromoles TE per g of FW from the average of triplicate determinations.

Oxygen radical scavenging capacity (ORAC) assay

Briefly, 25 µL of Trolox standard or pomegranate juice in 75 mM potassium phosphate buffer, pH 7.4 (working buffer), was added in triplicate wells to a 96-well, black, clear bottom microplate. 150 µL of 0.96 µM fluorescein in working buffer was added to each well and incubated at 37 °C for 20 min, with intermittent shaking. After incubation, 25 µL of freshly prepared 119 mM 2,2'-azobis(2-amidinopropane) dihydrochloride (ABAP) in working buffer was added to the wells using a 12-channel pipetter. The microplate was immediately inserted into a Synergy™ HT plate reader (Biotek Instruments, Winooski, VT) at 37 °C. The decay of fluorescence at 528 nm was measured with excitation at 485 nm every minute for 60 min. Quantification was based on the standard curve generated with Trolox, and average results from triplicate analyses were reported as micromoles TE per g of FW (Prior et al., 2003).

Major organic acids

The pomegranate juice (1 mL) was diluted with 5 mL of 1 M HCl. After flushing with nitrogen, the samples were centrifuged at 2000 rpm for 15 min and placed in a water bath at 90 °C for 30 min. The samples were cooled to room temperature, and the supernatant was filtered through a 0.45 µm membrane filter. A Hewlett-Packard (Avondale, PA) HP 1100 HPLC system with a diode array detector was used for organic acid analyses (Chen, En, & Zhang, 2006). An Agilent Zorbax Eclipse XDB-C18, 3.5 µm, 4.6 x 150 mm column was used with an isocratic mobile phase of 0.5% ammonium phosphate, pH adjusted to 2.8 with phosphoric acid. The flow rate was 0.5 mL/min and the injection volume was 20 µL. The column temperature was maintained at 40 °C and the detection was done at 214 nm. All the measurements were in triplicate and averages were reported as mg/100g of FW based on the external standards (10-1600 µg/mL).

Major Sugars

The aril juice was diluted with water and centrifuged at 2000 rpm for 15 min and filtered through a 0.45 µm membrane filter and injected into a Agilent (Santa Clara, CA) HP 1260 Infinity HPLC system connected to a Sedex 85 Evaporative Light Scattering Detection system (ELSD) (Richard Scientific Novato, CA). A Beckman µ-Spherogel 300 x 7.5 mm carbohydrate column was used at 80 °C. The mobile phase was water at a flow rate of 0.6 mL/min and the injection volume was 15 µL. The sugars were identified by comparison of their retention times with pure external standards and quantified using standard curves generated with the external standards. Triplicate measurements were made and average results reported as mg/100g FW (Martens & Frankenberger, 1991).

Major phenolic compounds

Pomegranate juice was diluted, centrifuged and filtered through a 0.45 μm membrane filter. The samples were injected into a Hewlett-Packard HP 1100 HPLC system equipped with a diode array detector. The separation column was a Beckman Ultrasphere C18, 5 μm, 4.6 x 250 mm, with temperature maintained at 40 °C. The mobile phase consisted of solvent A, methanol/acetic acid/water (10:2:88, v/v/v); solvent B, acetonitrile; and solvent C, water at a flow rate of 1 mL/min and injection volume of 20 μL. A linear gradient was used as follows: at 0 min, 100% solvent A; at 5 min, 90% solvent A and 10% solvent B; and at 25 min, 30% solvent A and 70% solvent B, with a 5 min postrun of 100% solvent C. A postrun was carried out to clean and prevent column build up between sample runs. Detection was carried out at 260 (quercetin, ellagic acid, punicalagin), 280 (catechin, epicatechin, gallic acid), and 320 nm (caffeic, ferulic, *p*-coumaric acid). Identification was based on retention times and characteristic UV spectra with authentic standards. External standard curves were used for quantification. All analyses were performed in triplicate, and average values were reported (Pastrana-Bonilla, Akoh, Sellappan, & Krewer, 2003).

Statistical analysis

All samples were analyzed in triplicate, and the results are expressed as average ± standard deviation. All statistical analysis were conducted using one-way ANOVA and Duncan's multiple-range test was used to determine statistically significant differences of variables at $p \leq 0.05$ (SAS 8.2, SAS Inst., Inc., 1999). Correlation studies and their significance were performed using Pearson tests with Microsoft Excel software package (Microsoft Corp., Redmond, WA).

Results and discussion

Yield

The yield (% FW) of pomegranate juice obtained by the two extraction methods are shown in Fig. 3.1a. Cultivar Cranberry had significantly ($p \leq 0.05$) higher yields by both blender (41.26%) and mechanical press (36.31%) methods. Across all cultivars and extraction techniques, juice yield varied from 17.1 - 41.26% based on whole fruit fresh weight. However, in all the cultivars, the blender gave a better yield compared to the mechanical press. The dry matter contents of different cultivars are shown in Fig. 3.1b. The average dry matter content for blender was 10.73% and 9.56% for mechanical press extraction. The highest significant ($p \leq 0.05$) dry matter content was found in cultivar White Don Wade (12.47% of FW) by blender (Fig 3.1c).

Total polyphenols, antioxidant capacity and anthocyanin composition

Pomegranate juice has high levels of phenolic acids, flavonoids and other polyphenolic compounds which contribute to its good antioxidant capacity and as an effective scavenger of several reactive oxygen species (Aviram, Fuhrman, Rosenblat, Volkova, Kaplan, & Hayek, 2002; Kulkarni & Aradhya, 2005). The amount of total polyphenols (TPP) varied between 28.88 - 85.84 mg GAE/100 g FW) (Fig. 3.2a). Among the cultivars, Cranberry had the highest significant ($p \leq 0.05$) concentration of TPP (85.84 mg GAE/100 g FW) in the fruit juice obtained using blender and cultivar Afganski (67.42 mg GAE/100 g FW) in the fruit juice obtained using mechanical press. These values were in accordance with previous studies on pomegranate by Pande & Akoh (2009) and Gil et al., (2000). However, they were lower compared to the values reported from pomegranate arils widely grown in Turkey (Özgen et al., 2008; Çam, Hışıl, &

Durmaz, 2009). These variations are likely due to the differences among cultivars, growing seasons, agricultural practices and variations in the applied total polyphenolic assays (Çam et al., 2009).

FRAP, TEAC and ORAC methods were used to test the antioxidant capacities of pomegranate juice. Our results showed that the antioxidant capacity among cultivars averaged 21.37, 9.07 and 611.97 μM TE/g of FW by the FRAP, TEAC and ORAC methods, respectively, for blender; 15.68, 7.64 and 593.78 μM TE/g FW, respectively, for mechanical press (Fig. 3.3). For blender, the highest significant ($p \leq 0.05$) FRAP value was found in Cranberry (38.57 μM TE/g FW), Afganski (38.54 μM TE/g FW) and Nikitski ranni (35.39 μM TE/g FW), highest TEAC value was Mejhos (11.03 μM TE/g FW) and highest ORAC value was Eve (693.95 μM TE/g FW). Cultivar Afganski had the highest significant ($p \leq 0.05$) FRAP value (24.42 μM TE/g FW), Cranberry had the highest TEAC value (10.59 μM TE/g FW) and Kaj-acik-anor had the highest ORAC value (652.36 μM TE/g FW) for mechanical press. The FRAP values were higher compared to TEAC values and they were similar to previous published results (Pande & Akoh, 2009).

The red-pink color of pomegranate juice may be attributed to a class of water soluble pigments known as anthocyanins which are high in antioxidant activity (Seeram & Nair, 2002). Cultivar Kaj-acik-anor with dark red aril color had the highest significant ($p \leq 0.05$) total anthocyanin content in the juice extracted with both blender (36.56 mg/100 g FW) and mechanical press (33.01 mg/100 g FW) (Fig. 3.2b).

The correlation coefficient (r) was significant ($p \leq 0.05$) between FRAP and TPP content ($r = 0.90$) for dark colored juices using the blender whereas a correlation of r =

0.65 was found for light colored juices (Table 3.1a). Similarly for mechanical press, correlation was found between FRAP and TPP content (r = 0.70) for dark colored juices. A positive correlation was found between TPP content and TEAC in light (r = 0.46) and dark juices (r = 0.51) obtained using blender, and in dark juice (r = 0.43) obtained using mechanical press. For ORAC and TPP, almost no correlation existed in light juice (r = -0.01), and a negative correlation was observed in dark juice (r = -0.66) with blender and (r = -0.62) mechanical press. These results suggest that the antioxidant capacity of pomegranate juice may be attributed to total polyphenols content (Pande & Akoh, 2009; Tzulker, Glazer, Bar-Ilan, Holland, Aviram, & Amir, 2007). In addition, the differences may be due to the different processing methods used which could affect the type and concentration of phenolics that are responsible for the antioxidant capacity of pomegranate juice (Çam et al., 2009). The phenolic content of juices obtained by pressing the arils in the laboratory was lower (1800 – 2100 mg/L) when compared to the commercial juices (> 2500 mg/L) as the industrial processing would extract some phenolic compounds from the fruit rind (Gil et al., 2000). Also, addition of ascorbic acid to commercial pomegranate juice accounts for higher antioxidant capacity (Pande & Akoh, 2009).

The total anthocyanin content was positively correlated with TPP for light juice (r = 0.48) from blender and light juice (r = 0.30) from mechanical press. However, they were negatively correlated to TPP content of dark juice from blender and mechanical press (r = -0.33; r = -0.30, respectively). Low positive and negative correlations were found between antioxidant capacity and total anthocyanin content (Table 3.1a). This suggests that the anthocyanins did not play a key role in the

antioxidant mechanisms with these tests (Çam et al., 2009). Shwartz et al. (2009) and Gil et al. (2000), also reported that the low correlations may be due to the presence of other compounds like hydroxycinnamic acids, in addition to anthocyanins which influence aril color and overall antioxidant capacity. Therefore, further studies are required to study the antioxidant potential of anthocyanins and its contribution to the antioxidant activity of pomegranate juice (Noda, Kaneyuki, Mori, & Packer, 2002). Temperature and season of harvest is also an important factor influencing the final anthocyanin content and aril color. Increased temperature will contribute to the degradation of anthocyanins and high oxidative stress which induce peroxidase activities (Shwartz et al., 2009; Borochov-Neori et al., 2011). Correlations (Table 3.1a) existed between the different antioxidant methods for the light juice obtained using the blender and mechanical press. However, significant ($p \leq 0.05$) negative correlation was found between TEAC and ORAC methods, suggesting that more than one type of antioxidant capacity measurement are necessary to explain the various mechanisms of antioxidant action. Since, antioxidants perform a variety of functions, their activity and mechanisms are related to the composition and conditions of the antioxidant capacity test system (Prior & Cao, 1999).

Major organic acids and sugars

The flavor quality of pomegranate fruits is dependent on the levels and ratio of sugars and organic acids present (Özgen et al., 2008). The major sugars found in pomegranate juice were glucose and fructose as shown in Fig. 3.2c. The fructose content of juice was higher than glucose content in all the cultivars with the highest in White Don Wade cultivar using blender (58.30 mg/mL) and mechanical press (55.44 mg/mL). The fructose content was in the range between 22.81 - 58.30 mg/mL for blender and 22.48 - 55.44

mg/mL for mechanical press. The glucose content varied between 11.94 - 47.78 mg/mL in blender and 10.70 - 45.59 mg/mL in mechanical press. These results are similar to the previously reported results for pomegranate cultivars grown in Turkey (Tezcan et al., 2009). The levels of glucose and fructose were relatively similar in juices obtained from the two juicing methods. Previous published results suggest higher glucose than fructose (Gabbasova & Abdurazakova, 1969) in Russian pomegranates and higher fructose than glucose in 40 Spanish cultivars (Melgarejo, Salazar, & Artes, 2000). These variations may be attributed to the different agricultural and soil conditions of the countries where they were grown.

The major organic acids found in different pomegranate cultivars are shown in Table 3.2. The organic acid content is responsible for the flavor of the juice, sensory quality, and possible health benefits. It also determines the freshness or spoilage of the juice (Aarabi, Barzegar, & Azizi, 2008). The microbial growth rate is also determined by the level of organic acids which in turn influence the quality of juice and its shelf life. Citric acid was the predominant organic acid found in all the cultivars extracted with blender (Table 3.2a) and mechanical press (Table 3.2b). Citric acid accounted for approximately 49.48% of the total acids quantified in the majority of the cultivars. These results are in accordance with previous published results of cultivars in Iran (Aarabi et al., 2008) and Georgia (Pande & Akoh, 2009). However, malic acid was the predominant acid, followed by citric acid in some of the Spanish cultivars (Legua, Melgarejo, Martnez, & Hernàndez, 2000). Citric acid ranged between 173.42 - 381.29 mg/100 g FW with an overall mean concentration of 261.39 mg/100 g FW for blender and 209.25 mg/100 g FW for mechanical press. Malic acid had an overall mean concentration of

188.73 mg/100 g FW and 152.07 mg/100 g FW for blender and mechanical press, respectively. The average levels of tartaric, succinic, ascorbic and oxalic acids for blender were 20.00, 48.87, 6.36 and 4.42 mg/100 g FW, respectively; for mechanical press, they were 14.03, 37.29, 4.78 and 2.80 mg/100 g FW, respectively. The overall mean content of succinic acid was slightly similar to the overall means reported by Poyrazoğlu, Gökmen, & Artík (2002) and Aarabi et al. (2008). However, no succinic acid was found in pomegranate cultivars grown in Spain (Melgarejo et al., 2000; Legua et al., 2000). Tartaric acid content was similar to the range reported by Melgarejo et al. (2000), but lower compared to the results reported by Poyrazoğlu et al. (2002). Ascorbic acid levels were similar to the ones previously reported (Aarabi et al., 2008) but, oxalic acid levels were lower than the ones previously published (Poyrazoğlu et al., 2002). However, the organic acid contents were low compared to the results published by Pande & Akoh (2009) suggesting that the distribution of organic acids in pomegranate fruits varied and depends on the sourness/sweetness of the cultivar.

Phenolic compounds profile

Table 3.3 shows the different concentrations of phenolic compounds found in different pomegranate cultivars extracted with blender and mechanical press, respectively. A variety of phenolic compounds were identified in the samples which primarily consisted of hydrolyzable tannins like gallic acid, ellagic acid, and punicalagin; phenolic acids such as caffeic, *p*-coumaric, and ferulic acids; and flavonoids such as catechins, epicatechin, and quercetin. The overall mean concentrations of phenolic compounds were as follows: for blender (Table 3.3a), gallic acid 159.19, catechin 64.01, epicatechin 21.72, caffeic acid 21.51, *p*-coumaric acid 6.00, ferulic acid 1.85, ellagic acid

30.79, punicalagin 140.63, quercetin 17.70 mg/100 g FW; for mechanical press (Table 3.3b), gallic acid 108.25, catechin 45.64, epicatechin 12.73, caffeic acid 18.91, *p*-coumaric acid 4.78 mg, ferulic acid 1.50, ellagic acid 22.72, punicalagin 82.13, quercetin 16.53 mg/100 g FW. Distinct intervarietal differences were seen in the phenolic acid composition of aril juice. Cultivar Cranberry had the highest (758.82 mg/100 g FW) total polyphenols in blender extracted juice and cultivar Nikitski ranni (431.99 mg/100 g FW) in mechanical press extracted juice. By visual comparison, the dark colored juices corresponded to higher level of total polyphenols. The presence of caffeic, ferulic, *p*-coumaric, gallic, ellagic acids, catechins, epicatechin, punicalagin, and quercetin in pomegranate juice have also been previously reported (Pande & Akoh, 2009; Poyrazoğlu et al., 2002; Artik, Murakami, & Mori, 1998). However, the overall content of punicalagin, ellagic acid and quercetin in the juice was very low in comparison to the concentrations found in the peels. Gil et al. (2000) reported that the juice obtained in the lab by pressing the arils alone had 10 times lower punicalagin content than commercial pomegranate juices (1500 – 1900 mg/L). Therefore, the high concentrations may be attributed to the different processing conditions used in the industry, in addition to varying temperatures, pressing pressures and inhibition of enzymes. The phenolic compound profile of different cultivars will help in improved understanding of the significant contribution of different compounds towards the health benefits of pomegranate juice.

Comparison between blender and mechanical press

Table 3.1b shows the different values for the various analyses conducted on the pomegranate juice extracted using two different methods. Statistically significant

differences ($p \leq 0.05$) were observed in almost all the analyses except for ORAC, total monomeric anthocyanins and total sugars. Similar results have previously been reported, where the anthocyanins were relatively stable and no change in sugar composition was seen (Miguel, Dandlen, Antunes, Neves, & Martins, 2004). The results of this study suggest that the processing method applied for juice extraction significantly affects the chemical properties of the juice. It should be noted that the blender always had a higher antioxidant capacity and phenolic content when compared to the mechanical press. This may be due to the incorporation of seeds and pith while juicing, and these contribute to the antioxidant capacity of the juice. However, the most economical and easy method to juice the fruit is to use the whole fruit and apply the necessary hydrostatic pressure to release the juice from the arils. This method is used commercially and the bitterness from the peel is masked by having additional treatments and blending of some fruit juices (Miguel et al., 2004). The antioxidant capacity of commercial pomegranate juices is three times higher than a green tea infusion and red wine (Gil et al., 2000), mainly due to the presence of hydrolyzable tannins like punicalagin in the peel which have health promoting properties.

Conclusion

The results of this study demonstrate that the use of a blender will result in higher juice yield and greater antioxidant capacity compared to the mechanical press. This might be due to the incorporation of the seeds and pith which contribute to the antioxidant capacity. The sugar, organic acid and, total anthocyanin contents did not differ significantly between the two processing methods suggesting their stability during extraction. Significant correlations ($p \leq 0.05$) existed between total polyphenols and

FRAP method in dark color juice from blender. Overall, cultivar Cranberry, showed good juice characteristics based on total polyphenol content and antioxidant capacity. However, further studies are required to understand the influence of climate, agricultural practices and ripening season on the juice characteristics. Analyzing the different pomegranate cultivars in Georgia, in terms of yield, antioxidant capacity, organic acid, and sugars content will enable breeders to selectively breed, propagate and commercialize certain cultivars in terms of phytonutrient and health beneficial compounds.

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Figure captions

Fig. 3.1 (a) Scheme for juice extraction. (b) Yield based on FW. (c) Dry matter content of cultivars. Values are the average of triplicates. Values with the same letter for each cultivar are not significantly different at $p \leq 0.05$

Fig. 3.2 (a) Total polyphenols, TPP. (b) Total monomeric anthocyanins. (c) Total sugars. Values are the average of triplicates. Values with the same letter for each cultivar are not significantly different at $p \leq 0.05$

Fig. 3.3 Antioxidant capacity by (a) FRAP, (b) TEAC, (c) ORAC assays. Values are the average of triplicates. Values with the same letter for each cultivar are not significantly different at $p \leq 0.05$

Table 3.1a Correlation matrix (Pearson test) conducted on data obtained from different analytical methods[a].

	Blender									
	Light color juice[b]					Dark color juice[c]				
	TPP	FRAP	TEAC	ORAC	total monomeric anthocyanins	TPP	FRAP	TEAC	ORAC	total monomeric anthocyanins
TPP	1	0.65	0.46	0.31	0.48	TPP	1	0.9*	0.51	-0.62	-0.33
FRAP		1	0.7	0.39	0.01	FRAP		1	0.37	-0.38	-0.42
TEAC			1	0.14	0.08	TEAC			1	-0.22	-0.36
ORAC				1	-0.10	ORAC				1	-0.12
total monomeric anthocyanins					1	total monomeric anthocyanins					1

	Mechanical press									
	Light color juice[b]					Dark color juice[c]				
	TPP	FRAP	TEAC	ORAC	total monomeric anthocyanins	TPP	FRAP	TEAC	ORAC	total monomeric anthocyanins
TPP	1	-0.12	-0.3	-0.01	0.3	TPP	1	0.7	0.43	-0.66	-0.3
FRAP		1	0.53	0.62	0.23	FRAP		1	0.66	-0.66	-0.19
TEAC			1	0.2	0.11	TEAC			1	-0.88*	-0.3
ORAC				1	-0.15	ORAC				1	0.64
total monomeric anthocyanins					1	total monomeric anthocyanins					1

[a] The r value of correlation is given and its significance ($p \leq 0.05$) identified by an asterisk

[b] Light color juice. cultivars - White Don Wade, Turk Don Wade, Haku-botan, Don Sumner South Tree, Don Sumner North Tree, Entek Habi Saveh, Cloud

[c] Dark color juice. cultivars - Mejhos, Salavatski, Kaj-acik-anor, Nikitski ranni, Afganski, Eve, Cranberry

Table 3.1b Values obtained for various analyses using two different extraction methods[A]

Analyses	Blender	Mechanical press
Yield (%) FW	30.61 ± 5.20a	24.56 ± 4.41b
TPP (mg GAE/100 g FW)	57.41 ± 0.83a	45.00 ± 1.05b
Dry weight (%FW)	10.73 ± 0.16a	9.56 ± 0.08b
FRAP (µM TE/g FW)	21.37 ± 0.98a	15.68 ± 0.86b
TEAC (µM TE/g FW)	9.07 ± 0.59a	7.64 ± 0.58b
ORAC (µM TE/g FW)	611.97 ± 5.90a	593.78 ± 7.15a
Total monomeric anthocyanins (mg cyanidin 3-glucoside/100 g FW)	12.01 ± 2.11a	9.53 ± 1.04a
Total organic acids (mg/100 g FW)	525.83 ± 12.08a	424.21 ± 8.53b
Total sugars (mg mL^{-1})	75.00 ± 0.88a	68.34 ± 1.08a
Total polyphenols (mg/100 g FW)	437.45 ± 3.64a	339.23 ± 5.63b

[A]Values are the averages of triplicates ± standard deviation. Values with the same letter for each analyses in each row are not significantly different at $p \leq 0.05$

Table 3.2a Major organic acids in blender extracted juice (mg/100 g FW)[A]

Cultivar	Citric acid	Malic acid	Tartaric acid	Succinic acid	Ascorbic acid	Oxalic acid	Total acids
White Don Wade	277.48±5.66d,e	241.23±9.97b	13.83±0.74f,g	36.37±2.41d,e	4.51±0.20b,c	1.99±0.16f	575.16±16.48c
Turk Don Wade	182.64±1.93g,h	145.69±2.01g,h	12.81±0.43f,g	43.01±0.19c,d,e	4.60±0.03b,c	3.45±0.30d,e,f	384.21±8.09f
Haku-botan	276.92±4.58d,e	116.75±2.34h	12.94±0.52f,g	56.55±6.73b,c	6.22±0.11b,c	4.87±0.50c,d	470.83±7.09d,e
Don Sumner South Tree	264.02±4.97d,e	230.55±4.61b,c	27.81±0.44b	51.78±0.49c,d	6.08±0.20b,c	4.81±0.09c,d	585.06±10.02c
Don Sumner North Tree	330.55±9.94a	309.84±6.56b,c,d	15.95±0.47f,g	38.62±2.05d,e	5.30±0.09b,c	2.09±0.10f	701.74±18.40a,b
Mejhos	219.34±7.68b,c,d	196.93±3.47f,g,h	26.08±2.60b,c	39.85±0.11c,d,e	4.30±0.19c	4.62±0.55d	491.12±13.05d,e
Salavatski	226.75±1.22c,d,e	165.51±6.66f,g,h	19.80±2.88f,g	78.00±6.17a	5.57±0.86b,c	6.24±1.24b,c	498.03±11.35c,d
Kaj-acik-anor	340.39±5.95b,c	227.78±0.51b,c,d,e,f	16.93±1.01e,f	47.18±7.71c,d	4.03±0.15c	2.93±0.21e,f	632.23±15.54c
Nikitski ranni	381.29±7.97a	195.96±8.73b,c,d,e	27.48±1.68b,c	62.38±6.23a	14.37±3.93a	10.84±2.47a	686.79±17.42a
Afganski	196.88±4.20f,g,h	154.36±1.74f,g,h	23.50±0.19c,d	36.39±1.51d,e	7.00±0.27b	4.09±0.04d,e	418.55±10.00e,f
Entek Habi Saveh	232.07±6.63e,f	157.12±9.38e,f,g,h	20.68±0.45d,e	54.88±3.22c,d	4.39±0.37c	3.44±0.10d,e,f	466.49±14.46d,e
Eve	173.42±2.19d,e,f,g	155.98±3.47h	13.23±3.82f,g	26.60±2.10e	4.94±0.34b,c	3.95±0.76d,e	377.63±5.65f
Cranberry	348.56±7.58b	194.51±7.86b,c,d,e,f,g	37.10±6.51a	68.71±5.02a,b	13.94±2.62a	6.73±1.39b	669.54±16.22b
Cloud	209.21±8.83f,g	150.07±6.82c,d,e,f,g	11.92±0.53g	43.96±10.36c,d	3.81±0.09c	1.87±0.11f	404.24±5.39e,f

[A]Values are the averages of triplicates ± standard deviation. Values with the same letter for each cultivar in the same column are not significantly different at $p \leq 0.05$

Table 3.2b Major organic acids in mechanical press extracted juice (mg/100 g FW)[A]

Cultivar	Citric acid	Malic acid	Tartaric acid	Succinic acid	Ascorbic acid	Oxalic acid	Total acids
White Don Wade	241.55±4.70c	197.75±7.17b	11.31±0.34c,d	29.16±0.65d,e	4.25±0.28d,e,f	1.59±0.07d	485.8±12.30b,c,d
Turk Don Wade	179.86±4.45e,f	129.01±1.44d	11.11±0.09c,d	38.85±1.12c,d	3.69±0.12e,f,g	3.32±0.05b,c	373.82±3.23f,g,h
Haku-botan	248.72±4.81a,b,c	92.48±0.61e	9.52±0.24d	54.19±2.03a,b	6.19±0.21a,b	3.57±0.26b,c	418.09±6.38e,f
Don Sumner South Tree	214.78±3.88c,d	183.03±6.02b	12.41±0.12c,d	35.00±2.50c,d,e	5.32±0.13b,c	1.82±0.02d	452.36±6.05c,d,e
Don Sumner North Tree	238.27±3.91c	237.47±1.88a	12.60±0.18c,d	33.32±1.09d,e	4.69±0.22c,d,e	1.72±0.04d	528.69±6.56a,b
Mejhos	154.59±2.85f,g	141.40±2.39c,d	13.80±1.03c	23.65±2.67e	2.98±0.28g	2.26±0.22d	338.68±9.16i,h
Salavatski	185.19±4.57d,e	105.11±6.34e	15.97±2.96a,b	48.50±3.47b,c	5.14±0.27c,d	3.58±0.61d	367.33±8.35f,g,h
Kaj-acik-anor	188.78±6.41d,e	180.37±4.33b	10.73±3.92c,d	33.40±5.32d,e	3.97±0.64e,f,g	2.18±0.41d	426.45±15.48d,e,f
Nikitski ranni	296.38±5.26a,b	186.72±5.91b	21.95±1.73a	36.65±4.50a,b	7.12±0.62a	4.97±0.36a	559.32±7.88a
Afganski	135.38±3.25g	150.68±4.16c	18.19±2.82b	25.83±0.41d,e	6.99±0.25a	3.12±0.60c	343.87±7.62i,g,h
Entek Habi Saveh	228.05±5.84c,d	126.21±3.20c,d	12.95±0.84c,d	49.75±0.59b,c	3.44±0.26f,g	2.19±0.18d	428.67±6.12d,e,f
Eve	121.83±3.14g	127.48±3.75d	11.65±3.26c,d	22.25±0.63e	4.44±0.17c,d,e,f	3.36±0.85b,c	291.51±5.76i
Cranberry	297.22±8.06a	150.77±7.52c,d	22.72±3.50a	64.23±5.52a	5.05±1.55c,d	3.98±0.75b	543.97±16.50a,b,c
Cloud	199.01±6.76c,d,e	120.63±1.96d	11.55±1.28c,d	27.35±3.35d,e	3.70±0.78e,f,g	1.61±0.27d	380.46±8.13e,f,g

[A]Values are the averages of triplicates ± standard deviation. Values with the same letter for each cultivar in the same column are not significantly different at $p \leq 0.05$

Table 3.3a Individual phenolic compounds in blender extracted juice (mg/100 g FW)[A]

Cultivar	Catechin	Epicatechin	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Ellagic acid	Punicalagin	Quercetin	Gallic acid	Total polyphenols
White Don	30.84±1.41h	2.72±0.31f	20.27±0.01d,e,f,g	5.23±0.19f,g	1.44±0.00d,e	35.59±2.18a,b	25.28±3.01j	16.33±0.34c,d,e	92.73±2.30h	230.07±9.21g
Wade Turk Don	38.00±4.83g,h	20.55±1.93c,d	19.70±0.31e,f,g	4.82±0.12g	1.34±0.01e,f	34.89±2.35a,b	155.36±7.41e	16.43±0.90b,c,d,e	82.03±5.73i,h	361.33±1.64f
Wade Haku-botan	53.13±10.39e,f	19.25±2.40c,d	19.71±1.17e,f,g	4.72±0.45g	1.02±0.04f	25.99±7.29b,c,d	103.07±10.64g	23.09±1.58a	180.65±13.31d	410.67±4.68e
Don Sumner South Tree	67.16±9.46c,d,e	23.54±1.40c	22.18±2.97c,d,e,f	7.89±0.23c	2.45±0.09b,c	35.26±8.40a,b	133.47±10.46f	17.06±1.93b,c,d,e	154.42±10.07e,f	460.52±3.07c,d
Don Sumner North Tree	57.41±6.62d,e,f	11.01±2.18e	16.88±0.10g	6.83±0.20d	2.34±0.24c	43.11±13.11a	228.44±5.16b	16.56±0.70b,c,d,e	126.52±12.15g	494.98±3.33c
Mejhos	59.26±6.70d,e,f	17.00±0.88d,e	23.47±0.82b,c,d	5.75±0.06e,f	1.57±0.06d,e	27.60±3.57b,c,d	196.00±9.92c	15.86±1.37d,e	167.22±8.01d,e	411.22±2.26e
Salavatski	74.04±14.50c	19.72±4.20c,d	19.90±0.15e,f,g	5.87±0.54e	1.56±0.08d,e	31.19±10.95a,b,c	314.74±5.46a	18.18±1.12b,c,d	159.85±9.54e,f	642.18±1.54b
Kaj-acik-anor	67.19±5.08c,d,e	12.00±0.43e	18.63±2.48g,f	4.97±0.02g	1.33±0.07e,f	26.36±6.92b,c,d	235.54±12.79b	16.45±0.26b,c,d,e	66.84±2.60i	432.18±2.13d,e
Nikitski ranni	60.58±7.85c,d,e	34.14±1.67b	26.75±1.63a,b	8.49±0.21b	2.68±0.23b	36.38±5.87a,b	173.30±2.37d	18.70±1.45b,c	170.15±7.47d,e	495.94±1.79c
Afganski	45.55±3.50f,g	11.05±3.38e	18.00±5.26g	4.13±0.26h	1.76±0.41d	19.71±0.54c,d	58.82±6.10i	18.78±1.14b,c	142.49±9.91f,g	320.28±1.42f
Entek Habi Saveh	95.33±10.31b	24.99±1.56c	23.23±1.08c,d,e	5.14±0.69g	2.23±0.03c	32.53±9.83a,b,c	75.67±9.02h	18.26±1.11b,c,d	228.86±14.19b	439.04±2.66d,e
Eve	60.71±7.20c,d,e	37.22±5.92b	28.72±0.26a	6.20±0.19e	1.74±0.10d	25.89±5.05b,c,d	155.24±9.17e	18.00±1.73b,c,d	198.23±9.64c	451.14±2.31c,d,e
Cranberry	117.87±5.70a	57.89±7.21a	25.60±1.05a,b,c	10.25±0.37a	3.22±0.32a	41.11±4.99a	105.31±4.96g	18.92±2.03b	381.98±14.76a	758.82±2.92a
Cloud	69.09±4.00c,d	13.00±4.39e	18.23±0.67g	3.82±0.31h	1.27±0.25e,f	15.55±1.43d	8.58±4.87k	15.31±1.31e	76.80±11.88i,h	215.91±12.18g

[A]Values are the averages of triplicates ± standard deviation. Values with the same letter for each cultivar in the same column are not significantly different at $p \leq 0.05$

Table 3.3b Individual phenolic compounds in mechanical press extracted juice (mg/100 g FW)[A]

Cultivar	Catechin	Epicatechin	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Ellagic acid	Punicalagin	Quercetin	Gallic acid	Total polyphenols
White Don	29.03±5.66d,e	2.31±0.58d	19.33±1.82b,c,d	4.89±0.07c,d,e	1.62±0.02d	21.71±4.40b,c,d	13.51±4.81g	14.54±0.27d	58.67±6.40f	165.94±14.09f
Wade										
Turk Don	35.96±3.74d,e	9.44±1.41c	19.08±0.45c,d	4.79±0.20d,e,f	1.31±0.03e	18.91±4.68b,c,d	122.49±9.84b	14.50±0.60d	64.06±1.08f	302.34±2.15d
Wade										
Haku-botan	44.88±11.13b,c,d	16.93±5.00b	19.18±1.71b,c,d	4.47±0.42e,f	0.92±0.06f	20.83±2.60b,c,d	91.47±13.50c	21.85±2.16a	144.35±11.59b,c	384.84±4.23b,c
Don Sumner South Tree	58.57±10.71a,b	8.79±0.95c	19.89±0.25b,c,d	6.11±0.13b	2.45±0.37a	34.65±8.07a	82.55±10.27c,d	14.69±1.11d	139.02±10.15c	369.63±3.85b,c
Don Sumner North Tree	54.05±14.18a,b,c	7.50±0.41c	14.80±0.65g,f	5.31±0.22c	1.80±0.07c,d	36.48±13.61a	126.43±1.80b	16.24±0.66b,c,d	83.16±13.03e	359.92±3.39b,c
Mejhos	40.91±4.82c,d	10.62±1.98c	17.24±1.01d,e,f	3.64±0.34g	1.08±0.05e,f	14.87±2.68d	84.85±3.38c,d	15.24±1.03c,d	111.00±7.22d	401.96±6.71a,b,c
Salavatski	44.61±7.91b,c,d	16.31±3.05b	18.99±0.71c,d	4.41±0.24f	1.17±0.04e	29.48±6.75a,b	112.80±5.43b	18.10±0.68b	156.98±5.71a,b	405.72±1.96a,b
Kaj-acik-anor	65.66±10.52a	11.33±1.28c	18.13±1.52d,e	4.36±0.25f	1.31±0.06e	22.45±5.95b,c,d	154.04±1.20a	16.14±2.48b,c,d	55.41±3.99f	365.95±10.79b,c
Nikitski ranni	25.35±2.28e	15.87±2.06b	25.57±1.13a	6.35±0.35b	1.90±0.08c	26.90±4.86a,b,c	117.48±10.94b	17.35±1.90b,c	159.99±5.36a	431.99±1.92a
Afganski	30.76±1.90d,e	9.35±0.86c	13.21±0.46g	2.93±0.14h	1.24±0.07e	14.87±0.87d	31.70±4.70f	16.52±1.95b,c,d	92.68±6.66e	213.27±11.31e
Entek Habi	55.99±13.74a,b,c	17.69±4.22b	22.05±2.73b,c	5.07±0.14c,d	1.20±0.05e	16.37±2.66c,d	73.47±12.10d	16.27±0.81b,c,d	117.14±11.26d	392.46±3.27a,b,c
Saveh										
Eve	43.30±8.34b,c,d	17.62±3.69b	19.31±1.18b,c,d	4.37±0.33f	1.72±0.22c,d	22.30±1.80b,c,d	78.03±3.36c,d	17.45±0.92b,c	111.97±6.41d	396.90±2.76a,b,c
Cranberry	43.71±1.40b,c,d	23.74±2.42a	22.27±3.78b	7.56±0.25a	2.13±0.06b	26.46±2.52a,b,c	53.16±9.37e	17.51±1.20b,c	156.36±8.72a,b	356.21±1.89c
Cloud	66.28±1.05a	10.72±2.97c	15.82±1.77e,f,g	2.70±0.15h	1.22±0.10e	11.80±0.51d	7.94±5.90g	15.07±0.80c,d	64.82±11.03f	202.13±10.53e,f

[A]Values are the averages of triplicates ± standard deviation. Values with the same letter for each cultivar in the same column are not significantly different at $p \leq 0.05$

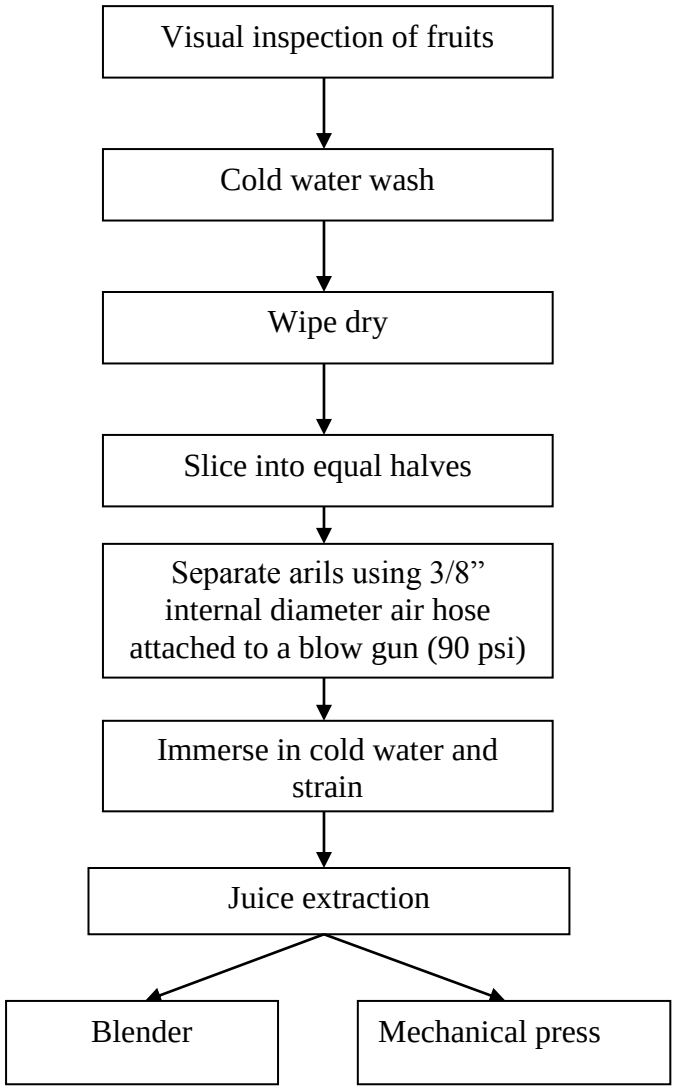
Visual inspection of fruits
Cold water wash
Wipe dry
Slice into equal halves
Separate arils using 3/8” internal diameter air hose attached to a blow gun (90 psi)
Immerse in cold water and strain
Juice extraction
Blender
Mechanical press

Figure 3.1a

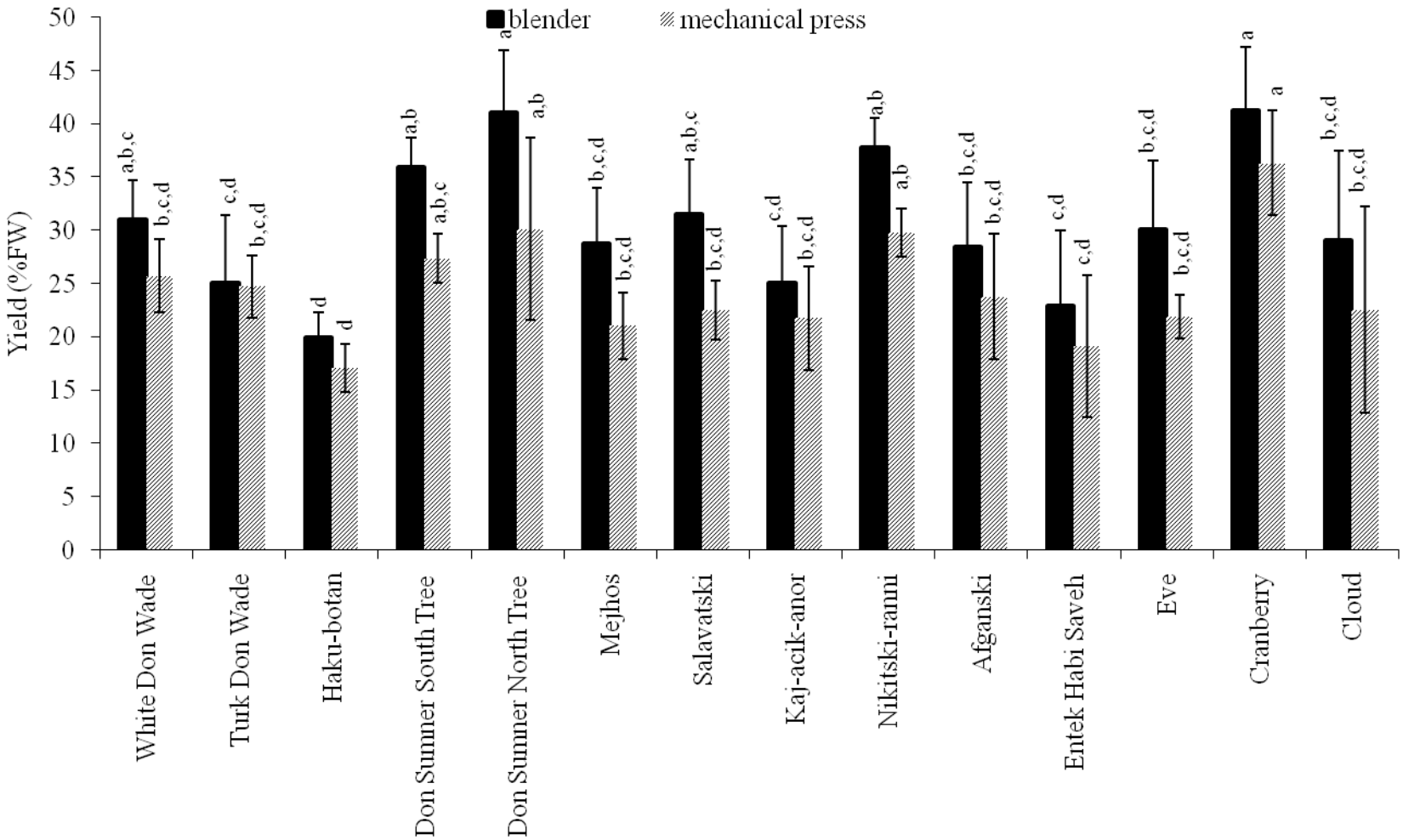
blender
mechanical press
Yield (%FW)
50
45
40
35
30
25
20
15
10
5
0
a,b,c
b,c,d
c,d
b,c,d
d
d
a,b
a,b,c
a
a,b
b,c,d
b,c,d
a,b,c
b,c,d
c,d
b,c,d
a,b
a,b
b,c,d
b,c,d
c,d
c,d
b,c,d
b,c,d
a
a
b,c,d
b,c,d
White Don Wade
Turk Don Wade
Haku-botan
Don Summer South Tree
Don Summer North Tree
Mejhos
Salavatski
Kaj-acik-anor
Nikitski-ranni
Afganski
Entek Habi Saveh
Eve
Cranberry
Cloud

Figure 3.1b

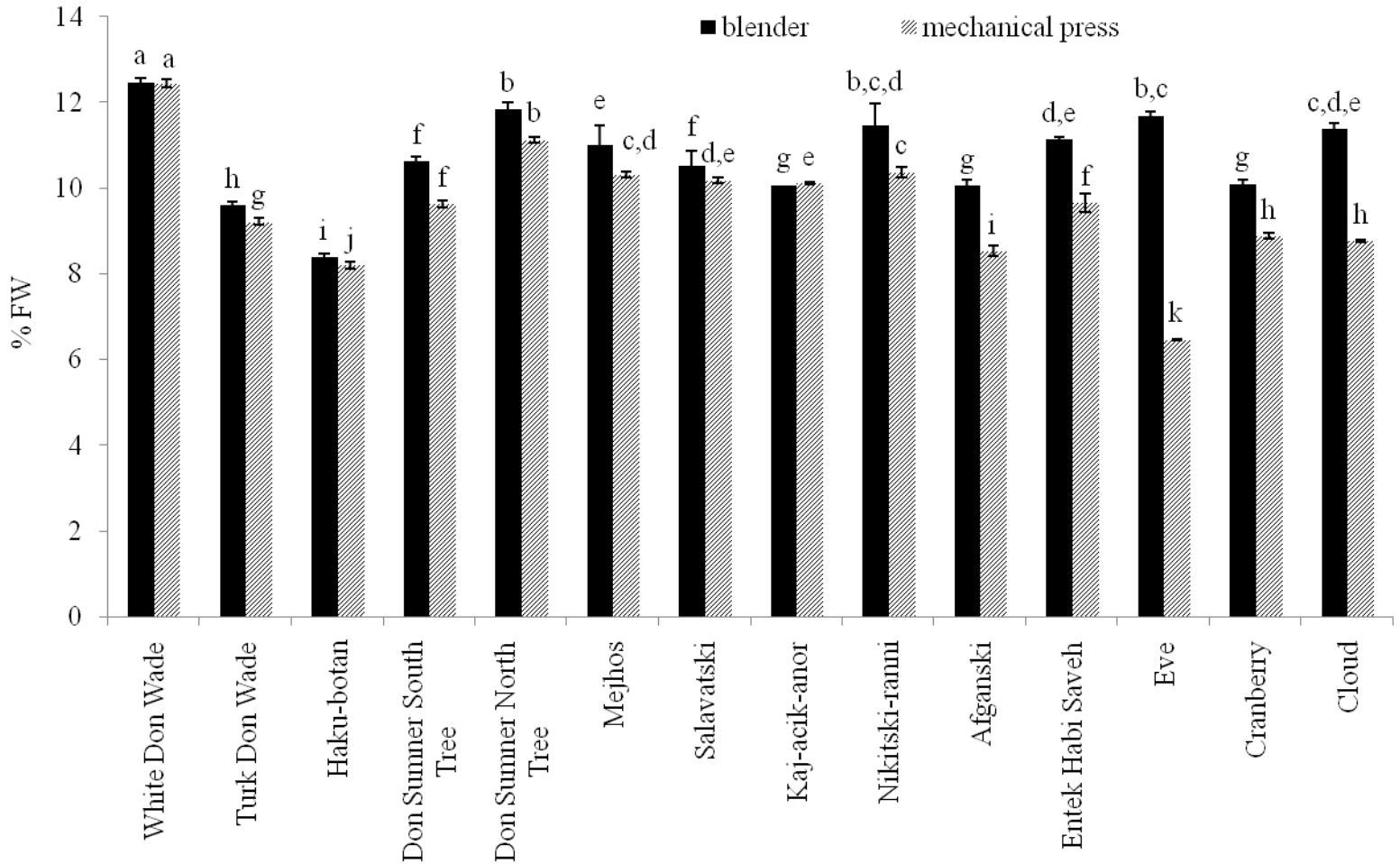
blender
mechanical press
% FW
14
12
10
8
6
4
2
0
a a
h g
i j
f f
b b
e c,d
f d,e
g e
b,c,d c
g i
d,e f
b,c k
g h
c,d,e h
White Don Wade
Turk Don Wade
Haku-botan
Don Sumner South Tree
Don Sumner North Tree
Mejhos
Salavatski
Kaj-acik-anor
Nikitski-ranni
Afganski
Entek Habi Saveh
Eve
Cranberry
Cloud

Figure 3.1c

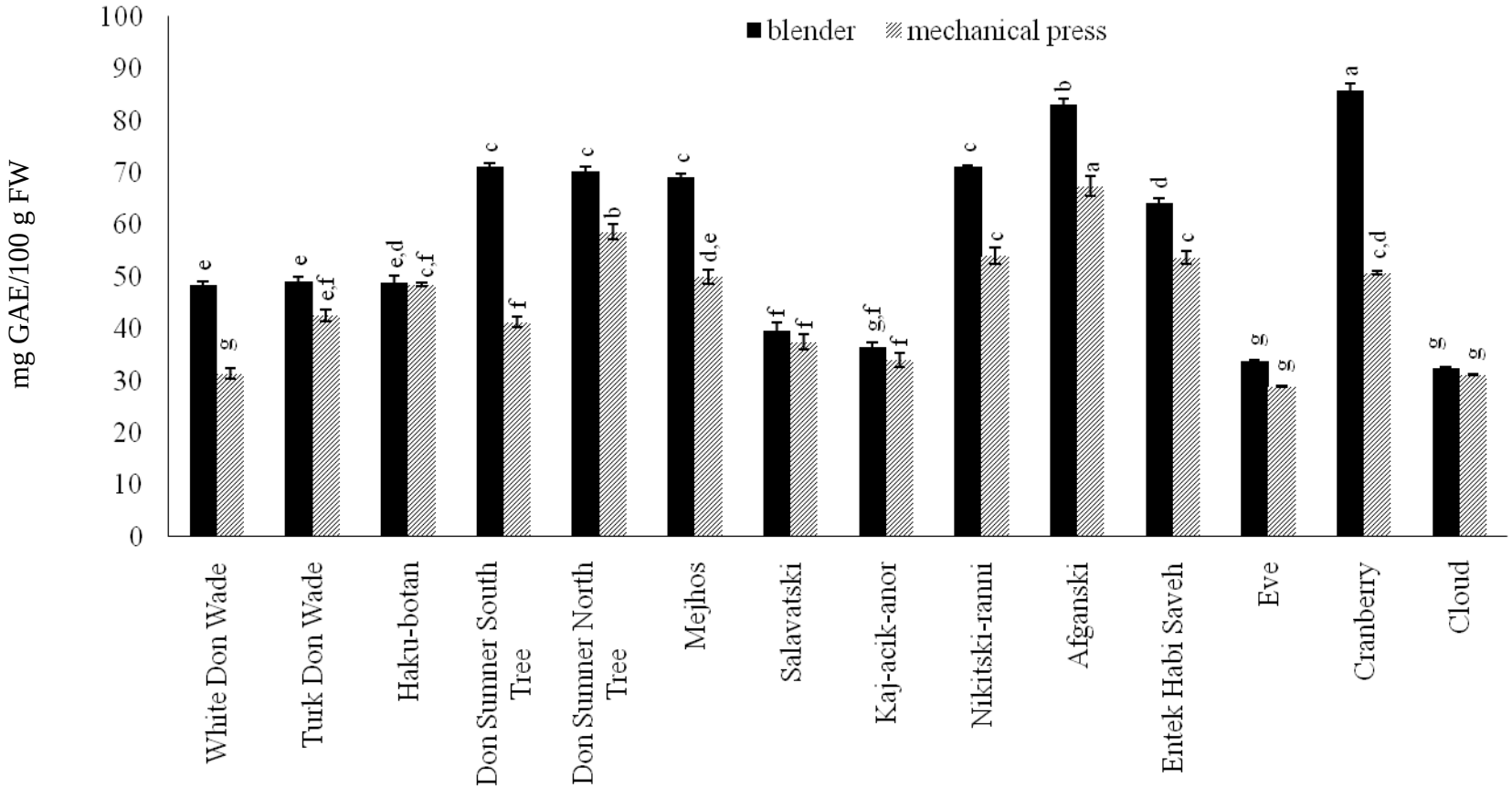
blender
mechanical press
mg GAE/100 g FW
100
90
80
70
60
50
40
30
20
10
0
White Don Wade
Turk Don Wade
Haku-botan
Don Summer South Tree
Don Summer North Tree
Mejhos
Salavatski
Kaj-acik-anor
Nikitski-ranni
Afganski
Entek Habi Saveh
Eve
Cranberry
Cloud

Figure 3.2a

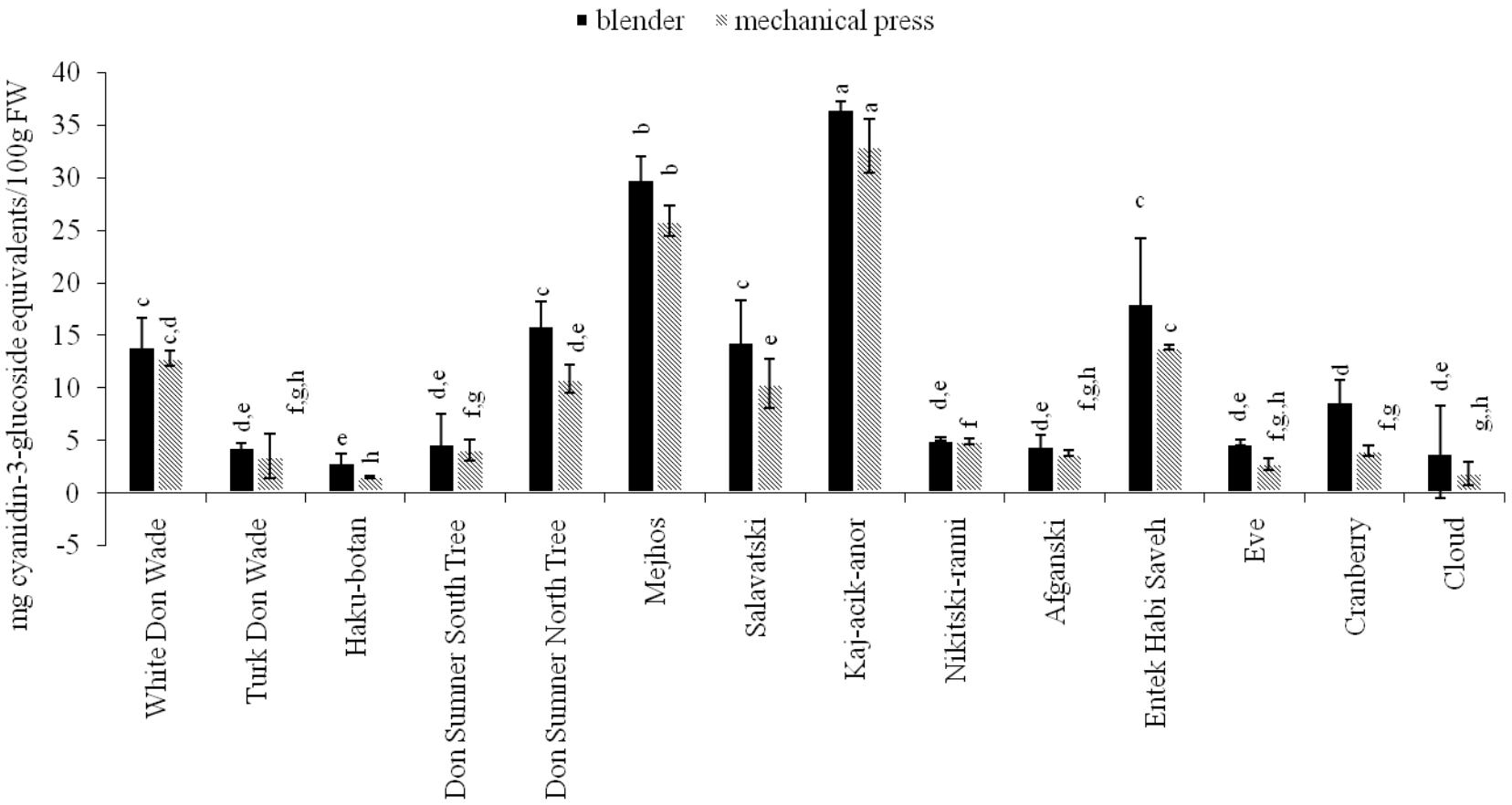
blender
mechanical press
mg cyanidin-3-glucoside equivalents/100g FW
40
35
30
25
20
15
10
5
0
-5
White Don Wade
Turk Don Wade
Haku-botan
Don Sumner South Tree
Don Sumner North Tree
Mejhos
Salavatski
Kaj-acik-anor
Nikitski-rannі
Afganski
Entek Habi Saveh
Eve
Cranberry
Cloud
c
c,d
d,e
f,g,h
e
h
d,e
f,g
c
d,e
b
b
c
e
a
a
d,e
f
d,e
f,g,h
c
c
d,e
f,g,h
d
f,g
d,e
g,h

Figure 3.2b

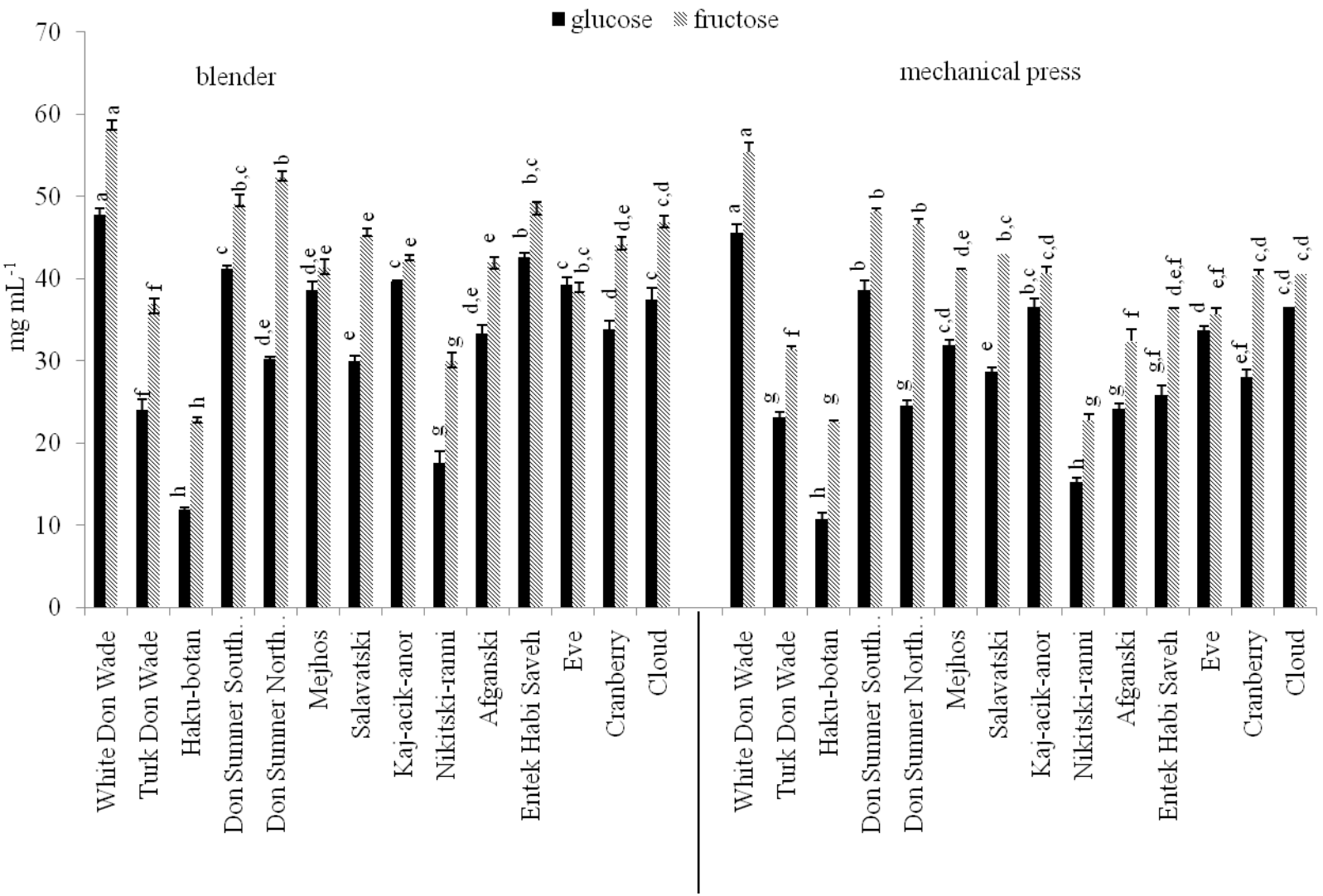
glucose
fructose
blender
mechanical press
mg mL-1
70
60
50
40
30
20
10
0
White Don Wade
Turk Don Wade
Haku-botan
Don Sumner South..
Don Sumner North..
Mejhos
Salavatski
Kaj-acik-anor
Nikitski-ranni
Afganski
Entek Habi Saveh
Eve
Cranberry
Cloud

Figure 3.2c

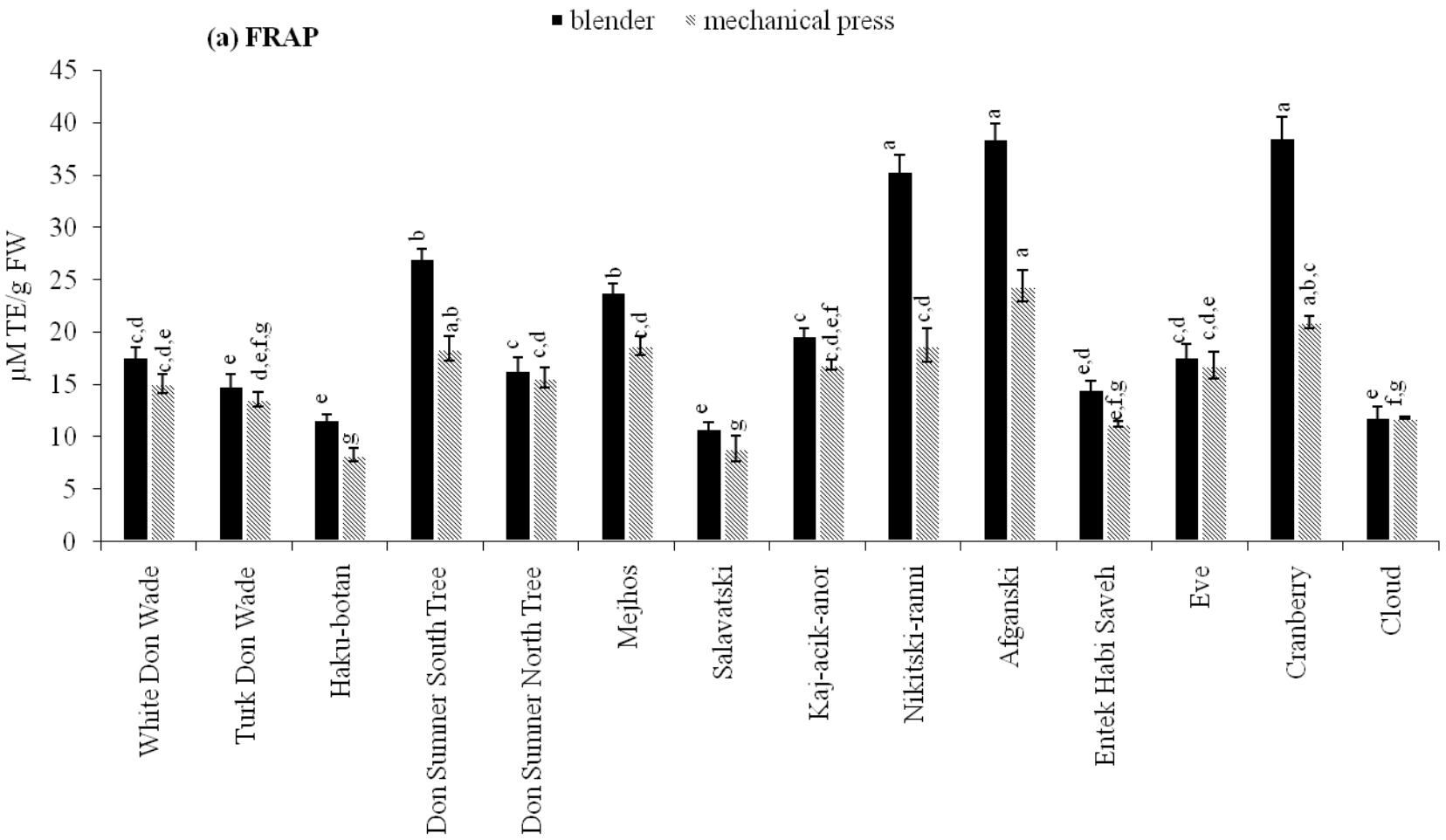
(a) FRAP
blender
mechanical press
µM TE/g FW
45
40
35
30
25
20
15
10
5
0
c,d
c,d,e
e
d,e,f,g
e
g
b
a,b
c
c,d
b
c,d
e
g
c
c,d,e,f
a
c,d
a
a
e,d
e,f,g
c,d
c,d,e
a
a,b,c
e
f,g
White Don Wade
Turk Don Wade
Haku-botan
Don Summer South Tree
Don Summer North Tree
Mejhos
Salavatski
Kaj-acik-anor
Nikitski-ranni
Afganski
Entek Habi Saveh
Eve
Cranberry
Cloud

Figure 3.3a

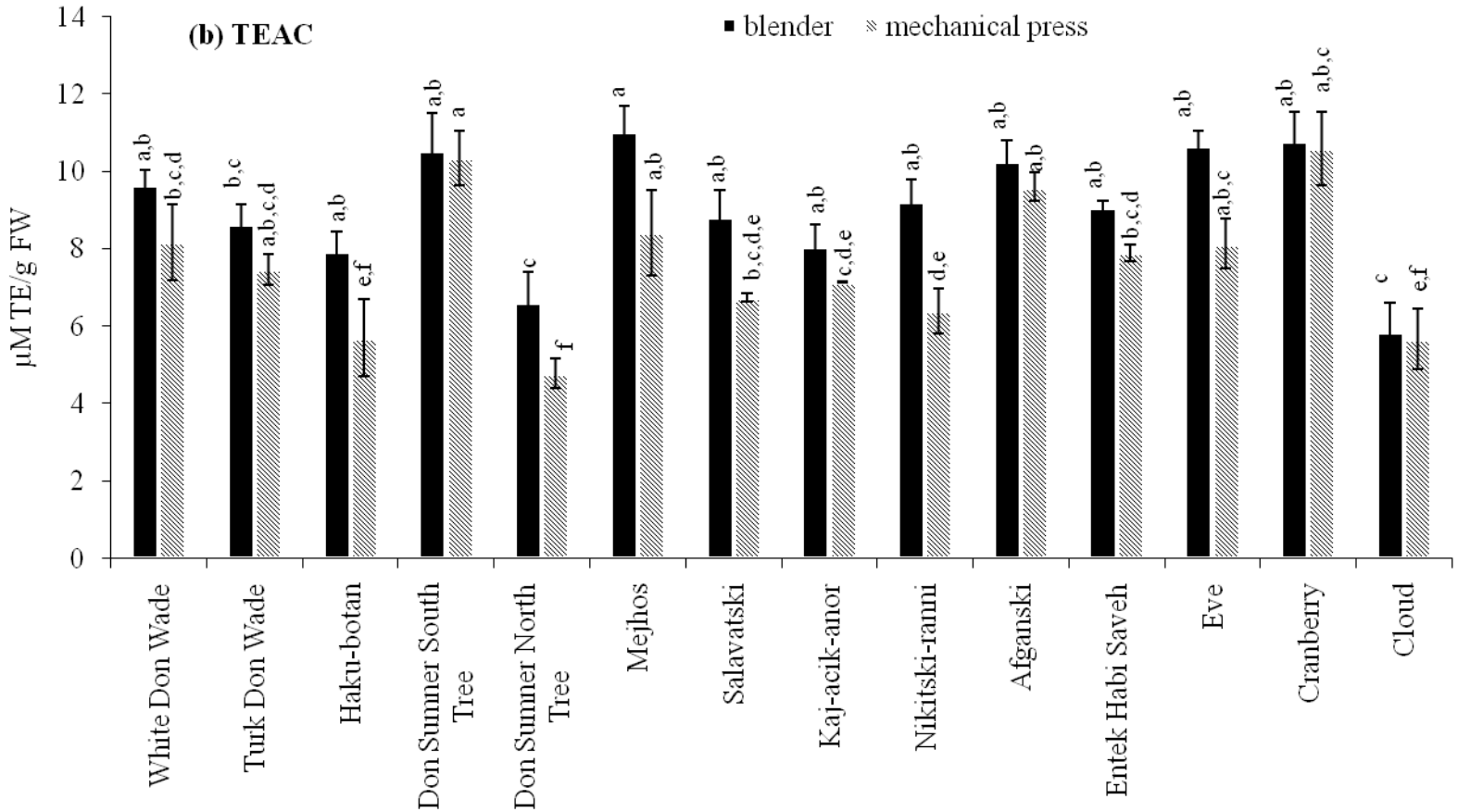
(b) TEAC
blender
mechanical press
µM TE/g FW
0
2
4
6
8
10
12
14
a,b
b,c,d
b,c
a,b,c,d
a,b
e,f
a,b
a
c
f
a
a,b
a,b
b,c,d,e
a,b
c,d,e
a,b
d,e
a,b
a,b
a,b
b,c,d
a,b
a,b,c
a,b
a,b,c
c
e,f
White Don Wade
Turk Don Wade
Haku-botan
Don Summer South Tree
Don Summer North Tree
Mejhos
Salavatski
Kaj-acik-anor
Nikitski-ranni
Afganski
Entek Habi Saveh
Eve
Cranberry
Cloud

Figure 3.3b

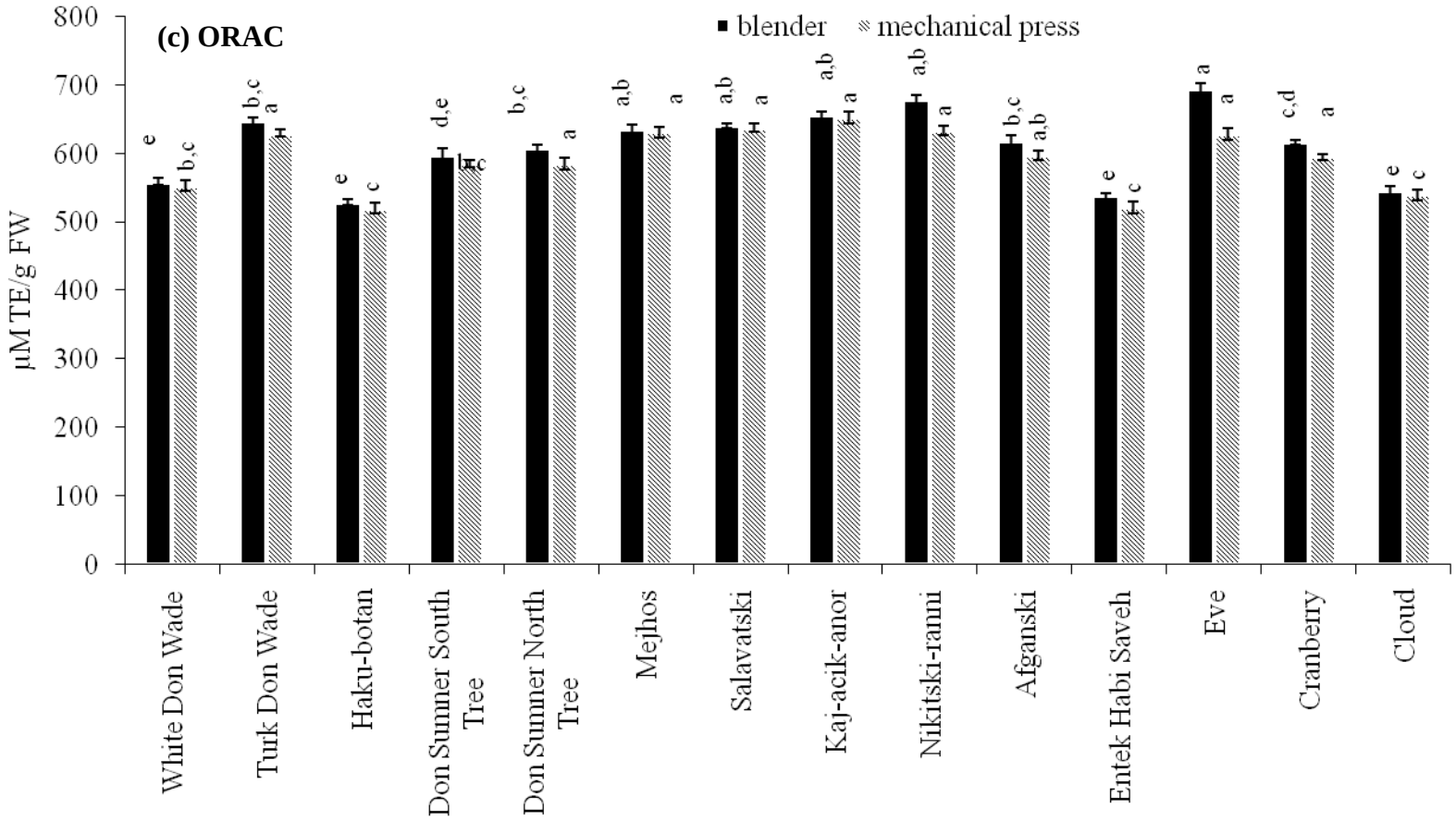
(c) ORAC
blender
mechanical press
µM TE/g FW
800
700
600
500
400
300
200
100
0
White Don Wade
Turk Don Wade
Haku-botan
Don Summer South Tree
Don Summer North Tree
Mejhos
Salavatski
Kaj-acik-anor
Nikitski-ranni
Afganski
Entek Habi Saveh
Eve
Cranberry
Cloud

Figure 3.3c

CHAPTER 4

PHYSICO-CHEMICAL CHARACTERISTICS OF JUICE EXTRACTED BY BLENDER AND MECHANICAL PRESS FROM POMEGRANATE CULTIVARS GROWN IN GEORGIA

Dhivyalakshmi Rajasekar, Casimir C. Akoh, Karina G. Martino, and Daniel D. MacLean

Reviewed and revision submitted to Food Chemistry on 12/5/11

Abstract

Pomegranate juice is consumed widely for its possible health benefits. The aril juice from fifteen pomegranate cultivars grown in Georgia were analyzed for juice yield based on fresh weight (FW) and physico-chemical properties using blender and mechanical press extraction. Blender had a significantly higher ($p \leq 0.05$) juice yield (42.04% FW) compared to mechanical press (38.05% FW). Total phenolics and antioxidant capacity was determined by Folin-Ciocalteau method and ferric reducing antioxidant power (FRAP), Trolox equivalent antioxidant capacity (TEAC), and oxygen radical absorbance capacity (ORAC) assays, respectively. Total monomeric anthocyanins were determined by pH differential method and RP-HPLC. The major anthocyanin was delphinidin 3-glucoside. High negative and significant ($p \leq 0.05$) correlations were found between pH and titratable acidity (TA). The total soluble solids content (TSS) averaged 15.59 in blender and 14.94 °Brix in mechanical press. Chemical analysis of juice showed significant differences ($p \leq 0.05$) among cultivars and extraction methods. Overall, blender was more efficient than mechanical press juice extraction.

Keywords: aril juice, extraction methods, yield, antioxidant capacity, total phenolics, anthocyanins, titratable acidity.

Introduction

The pomegranate (*Punica granatum* L.) fruit has been extensively used in folk medicine and is gaining popularity in recent times mainly due to its possible health benefits. These benefits may be attributed to the polyphenols which possess antioxidant activities and influence color, flavor, and texture (Poyrazoğlu, Gökmen, & Artík, 2002). The juice consists of antioxidative phenolics like punicalagins, hydrolyzable tannins, anthocyanins and ellagic acids (Gil, Tomas-Barberan, Hess-Pierce, Holcroft & Kader, 2000). Numerous studies suggest that these phenolic compounds can be used for the prevention and treatment of diseases like cancer and chronic inflammation (Lansky & Newman, 2007). Seeram et al. (2008) reported that the antioxidant activity of pomegranate juice is greater than other fruit juices and beverages.

Pomegranate fruit has been widely grown in Iran, Turkey, India, China, Afghanistan, Russia, and United States (Lansky et al., 2007). The edible fruit part is the arils which are consumed fresh or as processed products, predominantly as juice. The pomegranate juice contain six anthocyanin pigments namely 3-mono- and 3,5-diglucosides of cyanidin, delphinidin, and pelargonidin, which are primarily from the arils and responsible for the intense red color (Alighourchi, Barzegar, & Abbasi, 2008; Miguel, Dandlen, Antunes, Neves, & Martins, 2004). The evaluation of phenolic compounds and juice characteristics is essential to satisfy current market demands for quality fruit and for its potential use as a functional nutraceutical beverage. Studies have shown cultivar's significant influence on antioxidant activity and physicochemical properties like juice yield, pH, total soluble solids (TSS), titratable acidity (TA), total

phenolics, and anthocyanins (Mousavinejad, Emam-Djomeh, Rezaei, & Haddad Khodaparast, 2009; Özgen, Durgaç, Serçe, & Kaya, 2008; Ozkan, 2002).

The level of anthocyanin in the fruit depends on various factors, namely: species, varieties, growing conditions, seasonal variations, maturity index, processing methods, and storage conditions (Melgarejo, Salazar, & Artes, 2000; Ozkan, 2002). The effect of two different pomegranate juice extraction methods on anthocyanin stability was studied by Miguel et al. (2004). Gil et al. (2000) reported that the juice obtained from arils alone had lower antioxidant capacity than commercial juice obtained from whole fruit.

The purpose of this study was to evaluate and compare the juice yielding potential, antioxidant capacity, total polyphenols, total and individual anthocyanin levels of fifteen pomegranate cultivars grown in Georgia based on blender and mechanical press extraction methods.

Materials and methods

Plant material

Fifteen pomegranate (*P. granatum*, Punicaceae) cultivars grown in Georgia were used in this study. The cultivars Kaj-acik-anor, Rose, Don Sumner South Tree, Don Sumner North Tree, King, Crab, Thompson, Entek Habi Saveh, Afganski, Nikitski ranni, Fleshman, Haku-botan, Salavatski, Cranberry, and Pink were obtained from the University of Georgia Ponder farm, located near Tifton, GA. The trees at the Ponder Farm were planted in a loamy-sand soil (sand, 86%; silt, 7%; and clay, 7%) from 1990 to 1993. Orchard management was minimal until 2008, with no supplemental fertilizer or irrigation applied. Pruning was performed at irregular intervals after the initial planting. Fruits were harvested at maturity, as estimated based on soluble sugar content,

color, and total acidity, then transported to the University of Georgia Vidalia Onion Research Laboratory, where fruits were cooled to 7 °C prior to subsequent analysis.

Chemicals

The anthocyanin standards (cyanidin 3,5-diglucoside, cyanidin 3-glucoside, pelargonidin 3-glucoside), Folin-Ciocalteu reagent, 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), citric acid, and potassium persulfate were purchased from Sigma Chemical Co. (St. Louis, MO). Pelargonidin 3,5-glucoside was purchased from Fluka (Milwaukee, WI), delphinidin 3-glucoside and delphinidin 3,5-diglucoside was obtained from Extrasynthese (France). 2, 4, 6-Tripyridyl-s-triazine (TPTZ) and 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Acros Organics (Morris Plains, NJ). Other solvents and chemicals were purchased from Sigma Chemical Co., J. T. Baker Chemical Co. (Phillipsburg, NJ), and/ or Fischer Scientific (Norcross, GA).

Sample preparation

The fruits were washed with water and wiped completely dry. Fruits from each cultivar were then divided into equal portions for juice extraction with either an Oster® blender (Oster, Fort Lauderdale, FL) or hand operated juice extractor/mechanical press (Strite-Anderson Mfg.Co., Minneapolis, MN). In the blender, the pith, carpellary membrane and the arils were juiced, while in the mechanical press, it was only the aril juice (Fig. 4.1a). All sample preparation was done under dark conditions. The juice was flushed with nitrogen and stored at –80 °C until further analysis. All extractions were performed in triplicate.

Total polyphenols (TPP)

Total polyphenols were determined according to the Folin-Ciocalteu reagent method (Singleton & Rossi, 1965). To each 50 μL of extracted juice sample, 0.5 mL of Folin-Ciocalteu reagent and 1.5 mL of 7.5% sodium carbonate solution were added. The samples were then mixed well and allowed to stand for 30 min in the dark at room temperature. Absorption at 765 nm was read using a Shimadzu 300 UV-vis spectrophotometer (Shimadzu UV-1601, Norcross, GA). Quantification was based on the standard curve generated with 1-15 mg/L of gallic acid, and average results from triplicate determinations are reported as mg GAE/100 g FW.

Antioxidant capacity

Ferric reducing antioxidant capacity (FRAP) assay

The FRAP assay was performed according to the method of Benzie and Strain (1996) with minor modifications. Stock solutions of 300 mM acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine solution in 40 mM HCl), and 20 mM $FeCl_3.6H_2O$ were prepared. The FRAP reagent was prepared by mixing the stock solutions in 10:1:1 ratio and maintained at 37 °C and pH 3.6. Then, 10 μL of the sample and 300 μL of FRAP reagent were added into a 96-well microplate (Tsao, Yang, Xie, Sockovie, & Khanizadeh, 2005) and incubated at room temperature for 4 min. The absorbance was measured at 595 nm using a microplate reader (BioRad 680 XR, Hercules, CA). Trolox calibration solutions (100, 200, 400, 500 and 750 μM) were used to generate the standard curve and the results were expressed as micromoles Trolox equivalents (TE)/g FW. All assays were done in triplicate and averages were reported.

Trolox equivalent antioxidant capacity (TEAC) assay

The assay was performed based on the method of Lee, Kim, Kim, Lee, & Lee (2003) with slight modifications. Briefly 7 mM ABTS solution and 2.45 mM potassium persulfate solution were mixed and kept in the dark at room temperature for 12-16 h. The $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. To each 10 µL aliquot of Trolox standard or sample, 200 µL of diluted $ABTS^{\cdot+}$ was added , and the absorbance was read for 6 min at 734 nm using a microplate reader (BioRad 680 XR, Hercules, CA). The percent inhibition of absorbance was calculated and plotted as a function of Trolox concentration. TEAC values of samples were calculated from the standard curve and reported as micromoles TE/g FW from the average of triplicate determinations.

Oxygen radical scavenging capacity (ORAC) assay

Briefly, 25 µL of Trolox standard or pomegranate juice in 75 mM potassium phosphate buffer, pH 7.4 (working buffer), was added in triplicate wells to a 96-well, black, clear bottom microplate. 150 µL of 0.96 µM fluorescein in working buffer was added to each well and incubated at 37 °C for 20 min, with intermittent shaking. After incubation, 25 µL of freshly prepared 119 mM 2,2'-azobis(2-amidinopropane) dihydrochloride (ABAP) in working buffer was added to the wells using a 12-channel pipetter. The microplate was immediately inserted into a Synergy™ HT plate reader (Biotek Instruments, Winooski, VT) at 37 °C. The decay of fluorescence at 528 nm was measured with excitation at 485 nm every minute for 60 min. Quantification was based on the standard curve generated with Trolox, and average results from triplicate analyses were reported as micromoles TE/g FW (Prior et al., 2003).

Total monomeric anthocyanins (TMA)

The total anthocyanin content was estimated by the pH-differential (AOAC method 2005.02) using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M) on a UV-vis spectrophotometer (Shimadzu UV-1601, Norcross, GA). Samples were diluted in pH 1.0 and pH 4.5 buffers and then measured at 520 and 700 nm. The absorbance was calculated as $A = (A_{520nm} - A_{700nm})_{pH\ 1.0} - (A_{520nm} - A_{700nm})_{pH\ 4.5.}$

The monomeric anthocyanin pigment concentration was calculated as cyanidin-3-glucoside. The monomeric anthocyanin pigment (mg/L) = A x MW x DF x 1000/(ε x 1), where A = absorbance, MW = molecular weight (449.2), DF = dilution factor, and ε = molar absorptivity (26900). All measurements were done in triplicate and averages were reported.

Determination of individual anthocyanins by HPLC

Pomegranate juice was centrifuged and filtered through a 0.45 µm membrane filter. The samples were injected into a Hewlett-Packard (Avondale, PA) HP 1100 HPLC system equipped with a diode array detector. The separation column was a Beckman Ultrasphere C18, 5 µm, 4.6 x 250 mm, with temperature maintained at 40 °C. The mobile phases were solvent A, o-phosphoric acid/methanol/water (5:10:85, v/v/v), and solvent B, acetonitrile. The injection volume was 20 µL with a flow rate of 0.5 mL/min. The gradient followed was 100% solvent A at 0 min, 90% solvent A and 10% solvent B at 5 min, 50% solvent A and 50% solvent B at 25 min, with 5 min postrun with HPLC grade water. The anthocyanins were detected at 520 nm based on retention times and characteristic UV spectra. Individual external, authentic standards were used to construct

standard curves in the range of 0.5 – 15 µg/mL and used for quantification (Yi, Fischer, & Akoh, 2005). All analyses were performed in triplicate, and average values were reported.

pH and total soluble solids (TSS) content

The pH and soluble solids content of the juice were measured immediately after extraction using a pH meter (IQ240, IQ Scientific Instruments, Loveland, CO) and a digital refractometer (300034, SPER Scientific, Scottsdale, AZ), respectively. The refractometer was calibrated using distilled water and measurement was done with the temperature compensated mode. The soluble solids content was expressed as °Brix. All measurements were made in triplicate and average results reported.

Maturity index (TSS:TA) was calculated based on the classification made by Martinez, Melgarejo, Hernandez, Salazarm, and Martinez (2006).

Sweet varieties: MI = 31-98

Sour-sweet varieties: MI = 17-24

Sour varieties: MI = 5-7.

Measurement of aril juice color

The color of the aril juice was measured using a colorimeter (Chroma Meter CR-301, Minolta, Ramsey, NJ) (Solomon et al., 2006). The dimensions of 'L*', 'a*', 'b*', 'C' and 'H' were measured and the juice color index calculated according to the equation: (180 – H)/(L + C). L* represents lightness, a* redness, b* yellowness, C* chroma, and h° hue angle. Standardization of the instrument during each sample measurement was done using a black and a white tile (L = 91.10, a = -1.12, b = 1.26). The average values of triplicate measurements were reported.

Titratable acidity (TA) and formol number

The titratable acidity of the juice was measured using a pH meter (AOAC official methods of analysis, 1984), where the juice was titrated against 0.1 NaOH until the endpoint of pH 8.1. The values were expressed as percentage of citric acid. Formol number was measured using potentiometric titration of the juice against 0.1 N NaOH after the addition of formaldehyde till it reached end point pH of 8.1. It was expressed as mL of 0.1 N NaOH/100 mL sample (Anonymous, 1984). Triplicate measurements were obtained and the average values reported.

Statistical analysis

All samples were analyzed in triplicate and the results were expressed as average ± standard deviation. All statistical analysis were conducted using one-way ANOVA and Duncan's multiple-range test was used to determine statistically significant differences of variables at $p \leq 0.05$ (SAS 8.2, SAS Inst., Inc., 1999). Correlation studies and their significance were performed using Microsoft Excel software package (Microsoft Corp., Redmond, WA).

Results and discussion

Juice yield

High juice yield is a desired property for juice production. There was a significant difference ($p \leq 0.05$) between the blender and mechanical press methods of extraction. On average, the blender gave more juice yield (42.04% FW) compared to the mechanical press (38.05% FW). Cultivar Thompson gave the highest juice yield (51.16%) with blender, and cultivar King (45.29%) with mechanical press, both based on fresh weight (FW) of the fruits (Fig. 4.1b). The juice yield for Turkish pomegranates obtained from

laboratory press was 34.7% FW (Türkmen & Ekşi, 2011). Zarei, Azizi, and Bashiri-Sadr (2010) reported juice yield obtained using an electric extractor for cultivars grown in Iran between 48.02 - 63.52% FW and Martinez et al. (2006) reported juice percentage between 17.63 - 50.01% FW.

pH, Total soluble solids, titratable acidity

Some of the characteristics of the aril juice which determines its quality such as TSS, titratable acidity, pH, and formol number are listed in Tables 4.1a & 4.1b. The TSS levels in juice ranged from 13.80 - 16.57 °Brix. The minimum brix degree of pomegranate juice should be 14.0 according to AIJN proposal and it is dependent on factors like cultivar, year and region of growth, and maturity stage of the fruit (Anonymous, 2008). Cultivar Rose had the highest TSS content in blender (16.57 °Brix) and cultivar Kaj-acik-anor in mechanical press (15.83 °Brix). This shows that the fruits were at a fully ripe stage. Our results are similar to those reported by Martinez et al. (2006) for five Spanish cultivars, Tehranifar, Zarei, Nemati, Esfandiyari, & Vazifeshenas (2010) for cultivars grown in Iran. The "taste" of the juice is generally defined by the ratio of TSS:TA. The TA values varied from 0.13-2.97% citric acid. Cultivar Haku-botan had a very low TSS:TA ratio in blender (13.83:2.97) and mechanical press (13.80:2.56), indicating that it might be a sour cultivar. This was accompanied by the low pH value of the cultivar in blender (2.66) and mechanical press (2.50) extractions. With increase in maturity, the pH value increased with a maximum of 4.08 for cultivar Fleshman, in blender extracted juice. The pH values were in the range of 2.50 - 4.08 and similar to studies reported by Ozgen et al. (2008) and Mena et al. (2011). Formol number was between 0.60-1.40 mL 0.1N NaOH/100 mL. Our values were lower compared to that

reported by Poyrazoğlu et al. (2002) and Türkmen et al. (2011). The maturity index values showed wide ranges among the cultivars (Tables 4.1b & 4.1c). Based on these values, cultivars Don Sumner South Tree, Don Sumner North Tree, King, Thompson, Fleshman and Pink can be classified as sweet cultivars; Kaj-acik-anor, Rose, Nikitski ranni, Salavatski and Cranberry as sour-sweet cultivars; and, Crab, Entek Habi Saveh, Afganski and Haku-botan as sour cultivars. The most popular, cultivar Wonderful had maturity index values varying from 11 - 16 (Ben-Arie, Segal, & Guelfat-Reich, 1984) and is considered to be sour-sweet. The physico-chemical characteristics of the juice indicate a wide range of genetic diversity among the cultivars grown in Georgia. Genetic diversity have also been reported for the wild pomegranate collection of the Indian Himalayas and Tunisia (Narzary, Mahar, Rana, & Ranade, 2009; Jbir, Hasnaoui, Mars, Marrakchi, & Trifi, 2008).

There were significant correlations between maturity index (MI) and TA (Table 4.4a). No significant correlation existed between MI and TSS, suggesting that the ratio is mainly influenced by TA. Similar results have been reported (Mena et al., 2011). pH and TA were negatively correlated to each other ($p \leq 0.05$), whereas no significant correlations were found between pH and TSS.

Total polyphenols and antioxidant capacity

The phenolic compounds are formed in response to the reactive oxygen species (ROS) released by plants due to drought stress and are known to possess antioxidant activities like chelation of metal ions and quenching of free radicals (Gil et al., 2000).

The total polyphenolic content varied between 27.25 - 84.94 mg GAE/100 g FW (Table 4.1a). In both blender and mechanical press extracted juice, the highest significant

($p \leq 0.05$) total phenolic content was found in cultivar Entek Habi Saveh (84.94, 77.06 mg GAE/100 g FW), respectively. Cultivar Haku-botan had a very low total phenolic content in both blender (28.98 mg GAE/100 g FW) and mechanical press (27.25 mg GAE/100 g FW) extracted juice. Özgen et al. (2008), reported total phenolic content of six pomegranate arils grown in the Mediterranean region of Turkey that ranged between 1245 - 2076 mg/L. Our findings were similar to the ones previously reported by Pande & Akoh (2009). Gil et al. (2000) reported the total phenolic content of cultivar Wonderful from fresh arils as 2117 mg/L. The wide differences among different regions can be attributed to several factors including climate, growing region, type of cultivar, maturity, storage and processing methods (Melgarejo et al., 2000; Ozkan, 2002).

Determination of antioxidant capacity of juice helps in understanding the biological activities of the phenolic compounds responsible for improving human health and nutrition. Three methods (FRAP, TEAC, and ORAC) were used to evaluate the antioxidant capacities of the juice. FRAP and TEAC were electron transfer (ET) mechanism based assays and ORAC was based on hydrogen atom transfer (HAT) assay (Breksa & Manners, 2006). Significant differences ($p \leq 0.05$) were found among different cultivars using the Duncan test (Fig. 4.2) Cultivar Cranberry (42.30; 40.88 µM TE/g FW) had the highest significant ($p \leq 0.05$) FRAP value in blender and mechanical press, respectively. TEAC values were higher for cultivar Thompson (8.42 µM TE/g FW) for blender and cultivar Don Sumner North Tree (7.94 µM TE/g FW) for mechanical press extraction. For ORAC assay, cultivar Thompson showed high antioxidant capacity (1721.60 µM TE/g FW) for blender and cultivar Cranberry (1426.99 µM TE/g FW) for mechanical press. Similar results were published by Pande & Akoh (2009).

High positive and significant correlations (Table 4.4a) were found between antioxidant capacity (FRAP, TEAC, ORAC) and total polyphenols in light and dark juices obtained using blender and mechanical press. This suggests that the polyphenols contribute to the antioxidant activity. Similar results have been reported (Tzulker, Glazer, Bar-Ilan, Holland, Aviram, & Amir, 2007).

Positive and significant ($p \leq 0.05$) correlation was found between TEAC and ORAC methods in blender and mechanical press and FRAP and ORAC in mechanical press for light and dark color juices. This suggests that both methods are suitable to determine the antioxidant capacity of pomegranate aril juice. However, these results must be interpreted with caution as TEAC is an electron transfer (ET) based method, where the potential of the antioxidant to transmit one electron to reduce radicals is recorded. ORAC is a hydrogen atom transfer (HAT) method in which quenching free radicals by the antioxidant through hydrogen atom transfer is determined (Huang, Ou, & Prior, 2005). The mechanism of antioxidant actions is complex in a biological matrix and is influenced by several factors like the structure of the antioxidant, solvent system, and etc. Thus, more than one antioxidant test is needed to determine the different characteristics and reach a satisfactory conclusion (Prior, Wu, & Schaich, 2005).

Total monomeric anthocyanin and individual anthocyanins levels determined by RP-HPLC

The pomegranate juice has an attractive red color which serves as important criteria for quality of the juice and also for the marketability of processed pomegranate products. The total monomeric anthocyanin levels ranged between 0.40 - 41.97 mg cyanidin-3-glucoside equivalents/100 g FW (Fig 4.1c). By visual appearance, cultivar Kaj-acik-anor

produced dark red color juice, with a high total anthocyanin level for blender (41.97 mg cyanidin-3-glucoside equivalents/100 g FW) and mechanical press (31.30 mg cyanidin-3-glucoside equivalents/100g FW) extractions. Our results were comparable to previous studies (Gil et al., 2000; Tehranifar et al., 2010).

Low negative correlations were found between total monomeric anthocyanins and antioxidant assays (Table 4.4a). It was noticed that the cultivars which had the highest antioxidant capacity are those which had light pink arils. Cultivars having dark, red colored arils had low or intermediate antioxidant activity. This suggests that the anthocyanins are not significant contributors to the antioxidant capacity of the aril juice. Gil et al. (2000) also reported that only 6% of the antioxidant capacity of pomegranate juice was contributed by anthocyanins.

Six kinds of anthocyanins were separated from the aril juice by RP-HPLC: cyanidin 3-glucoside (Cya3), cyanidin 3,5-diglucoside (Cy3,5), delphinidin 3-glucoside (Dp3), delphinidin 3,5-diglucoside (Dp3,5), pelargonidin 3-glucoside (Pg3), and pelargonidin 3,5-diglucoside (Pg3,5) as shown in Fig. 4.3. The elution order of the anthocyanins were similar in all the cultivars, but the area under the peaks were significantly ($p \leq 0.05$) different. Delphinidin 3-glucoside was the major anthocyanin found in both blender and mechanical press (Tables 4.2a & 4.2b). Miguel et al. (2004) and Mousavinejad et al. (2009) reported that delphinidin 3-glucoside was the major anthocyanin found in cultivar "Assaria." In almost all the cultivars, the concentration of the diglucoside type anthocyanins were higher than monoglucosides. Pelargonidin 3-glucoside was not present in all the cultivars. The values obtained for anthocyanin profile of fifteen different cultivars in our study were lower compared to the results reported by

Gil et al. (2000), Alighourchi et al. (2008), and Mousavinejad et al. (2009). These differences may be due to the variations in cultivar, maturity of the fruits at the time of harvest, season of harvest, storage temperature and relative humidity (Miguel et al., 2004). The anthocyanin fingerprints of pomegranate cultivars are quite different and can be useful for various applications such as determination of juice authenticity.

A significant ($p \leq 0.05$) correlation existed between total monomeric anthocyanins determined by pH-differential method and total anthocyanins determined by HPLC for dark juice produced by blender ($r = 0.77$) and light juice produced by mechanical press ($r = 0.71$) as shown in Table 4.4a. No significant correlation was observed for the light juice produced with blender ($r = 0.14$) and dark juice with mechanical press ($r = 0.54$). In our study, we found that the total anthocyanin content obtained by pH-differential method was higher compared to HPLC. Similar results have been reported by Lee, Durst, & Wrolstad (2002) for blueberry juices. One of the reasons may be due to the different solvent systems used for HPLC and spectrophotometer which affect the spectral characteristics of the anthocyanins (Lee, Rennaker, & Wrolstad, 2008). Also, the maximum absorption value of different aglycons is varied. For example, pelargonidin absorbs at 520 nm and delphinidin at 546 nm, whereas their monoglucosides absorb at wavelengths which are 10-15 nm lower than the maximum absorption wavelengths of their respective aglycons (Giusti-Hundskopf, 1998). The other reason may be due to the presence of polymeric pigments in juice which contribute to the anthocyanin measurements in the spectrophotometer, and thus, have higher absorbance values. In HPLC system, these pigments are retained in the column and therefore may not add to the measured anthocyanin values (Lee et al., 2002). Even though the HPLC is

accurate and useful in measuring the anthocyanin levels, the pH-differential method is simple, rapid, economical, and has been verified by AOAC's validation guidelines (Lee et al., 2008).

Color values

For pomegranate juice, red color is an important characteristic for its quality. L* represents lightness, a* redness, b* yellowness, C* chroma and h° hue angle. Aril juice color was determined for the blender and mechanical press extracted juice (Tables 4.3a & 4.3b). Cultivars Don Sumner North Tree and Haku-botan had the highest L* value indicating that they have a lighter color. The a* and b* values were higher in cultivar Kaj-acik-anor showing that the red and yellow color components, respectively, were predominant in the aril juice. The purity or saturation of the color is defined by chroma value C*. Cultivar Kaj-acik-anor had the highest C* value for blender and mechanical press showing the presence of intense red color. The hue angle h° denotes the subtle distinction or variation in color (Wrolstad, Durst, & Lee, 2005). Cultivar Haku-botan had the highest value for both blender and mechanical press indicating a predominant yellow color. The color index values in our study were low compared to the results of Tzulker et al. (2007) and Shwartz et al. (2009). This may be due to the influence of climatic conditions and temperature changes which affect the color development of the pomegranates.

High positive correlations were present between total monomeric anthocyanins and a* in the dark juices obtained using blender and mechanical press. This can be related to the harvesting season of pomegranates, where low temperature and radiation resulted

in fruits with dark, red arils. The dark juices from blender and mechanical press had high positive correlations between total monomeric anthocyanins and C*.

Comparison between blender and mechanical press

Significant differences were seen between the methods for the different chemical analyses performed (Table 4.4b). ORAC, pH, titratable acidity, maturity index, a*, and total anthocyanins determined by RP-HPLC had no significant differences. The juice from blender is a combination of pith, carpellary membrane and seeds, whereas in the mechanical press, it is the juice from the arils. It was reported that the pith contains hydrolyzable tannins which consists of punicalagin isomers that may be responsible for about half of the total antioxidant capacity of the juice (Gil et al., 2000). However, it should be noted that the antioxidant capacity of commercial pomegranate juice is about 20-fold higher, as the peels contain a significant amounts of hydrolyzable tannins. In our study, based on the different antioxidant assays, the antioxidant levels of juice on an average was 1.13 times higher than the juice from mechanical press. Tzulker et al. (2007) reported that the juice from juice extractor had 3 times higher antioxidant levels when compared to the juice from arils. Significant differences in total monomeric anthocyanins between the two extraction methods may be due to the presence of polymeric pigments. High levels of polymeric pigment may be found in blender compared to mechanical press, mainly due to the degradation of anthocyanins and their reaction with tannins to form a complex (Wrolstad et al., 2005). HPLC determination of total anthocyanins was the sum of the individual concentrations of the various anthocyanins calculated based on their peak areas, whereas in pH-differential method, the values were based only on cyanidin 3-glucoside. This may explain the absence of significant differences detected for

total anthocyanins determined by RP-HPLC. Also, the anthocyanin content measured may be affected by the method, standard used for analysis, and processing techniques. Having no significant differences in pH, titratable acidity, and maturity index suggests that they are not influenced by the juicing methods. Similar results have been reported by Vàzquez-Araújo, Chambers, Adhikari, & Carbonell-Barrachina (2011).

Conclusion

This study shows statistically significant differences among the different pomegranate cultivars grown in Georgia in terms of yield, total phenolic content, antioxidant capacity and anthocyanin levels of the juice. When comparing the two methods used for juice extraction, the blender consistently had significantly higher yield, antioxidant capacity, total phenolic content and total monomeric anthocyanins than mechanical press. This may be due to the presence of seeds, pith and carpellary membrane which contributes to the antioxidant and phenolic content. No significant differences were observed in pH, titratable acidity, and maturity index suggesting that the method of juice extraction did not influence these chemical properties. Positive and significant correlations were found between the total phenolic content and FRAP, ORAC in light juice from both blender and mechanical press, and TPP and TEAC in dark juice from blender. Cultivar, Thompson with red to pink arils may be suitable for both fresh consumption and juice production based on yield, total polyphenols, antioxidant capacity and maturity index. Cultivar Kaj-acik-anor, a sour cultivar with dark red color arils and high anthocyanin content may be used for production of juice with good health benefits. The results of this study provide information about important physico-chemical properties of the juice which may enable

pomegranate growers in Georgia to select suitable cultivars to propagate for commercial cultivation and for the juice processing industry.

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Figure captions

Fig. 4.1 (a) Scheme for juice extraction. (b) Yield based on FW. (c) Total monomeric anthocyanins. Values are the average of triplicates. Values with the same letter for each cultivar are not significantly different at $p \leq 0.05$

Fig. 4.2 Antioxidant capacity by (a) FRAP, (b) TEAC, (c) ORAC assays. Values are the average of triplicates. Values with the same letter for each cultivar are not significantly different at $p \leq 0.05$

Fig. 4.3 Typical chromatogram showing the separation of individual anthocyanins by RP-HPLC at 520 nm of Kaj-acik-anor juice extracted using blender

Table 4.1a Total polyphenols[A]

Cultivar	Blender	Mechanical Press
Kaj-acik-anor	78.11±2.72e,f,g,h	56.46±0.88d
Rose	54.34±0.91d	35.09±0.68g,h
Don Sumner South Tree	57.18±3.98f,g,h	48.19±2.07e
Don Sumner North Tree	61.44±3.48e,f,g	57.49±2.69d
King	62.06±3.44e,f,g	41.39±3.00f
Crab	69.36±1.45h	45.67±3.44e,f
Thompson	64.85±5.27d,e	47.69±1.78e
Entek Habi Saveh	84.94±2.63a	77.06±3.21a
Afganski	71.14±4.31c,d	65.04±2.08c
Nikitski ranni	71.46±5.99c,d	71.36±2.74b
Fleshman	79.11±3.15a,b	74.12±4.08a,b
Haku-botan	28.98±3.65b,c	27.25±5.01c
Salavatski	64.35±4.64d,e,f	56.50±0.12d
Cranberry	65.50±0.31d,e	62.75±0.60c
Pink	61.30±2.77e,f,g	55.63±2.34d

[A] Values are the averages of triplicates ± standard deviation. Values with the same letter for each cultivar in the same column are not significantly different at $p \leq 0.05$.

Table 4.1b Characteristics of pomegranate juice extracted with blender[A]

Cultivar	pH	TSS (°Brix)	TA (%citric acid)	Formol no (mL 0.1N NaOH/100 mL)	Maturity index (TSS:TA)	Type	Aril color
Kaj-acik-anor	3.25±0.25d,e,f,g,h	16.43±0.23a,b,c	1.09±0.17d,e	1.13±0.32b,c,d,e,f	16.23±4.41g	Sour-sweet	Dark pink
Rose	3.72±0.3b,c,d	16.57±1.36a,b	0.55±0.02e,f,g	0.97±0.06c,d,e,f	50.97±3.71f	Sour-sweet	Light and dark pink
Don Sumner South Tree	3.85±0.17a,b,c	15.27±0.38b,c,d	0.22±0.02g	1.07±0.15b,c,d,e,f	90.31±5.59d	Sweet	Light cream
Don Sumner North Tree	3.53±0.05d,e,f	15.17±0.32c,d	0.23±0.03g	0.70±0.20e,f	98.23±1.96c	Sweet	Light pink
King	3.97±0.04a,b	15.83±0.31a,b,c	0.16±0.00g	0.93±0.21d,e,f	125.17±3.81a	Sweet	Light cream
Crab	3.14±0.17g,h	15.90±0.62a,b,c	1.19±0.44c,d	0.97±0.06c,d,e,f	15.49±4.79g,h,i	Sour	Dark pink
Thompson	3.98±0.12a,b	15.83±1.01a,b,c	0.16±0.00g	1.13±0.31b,c,d,e,f	106.35±3.77b	Sweet	Light pink
Entek Habi Saveh	3.33±0.03e,f,g	16.30±0.26a,b,c	1.97±0.68b	2.17±0.81a	12.66±1.06h,i	Sour	Light and dark pink
Afganski	2.96±0.15h	14.37±0.23d,e	1.56±0.51b,c	1.23±0.06b,c,d,e	10.08±1.27i,j	Sour	Light and dark pink
Nikitski ranni	3.59±0.03c,d,e	15.47±0.06a,b,c,d	0.76±0.19d,e,f	1.23±0.06b,c,d,e	22.20±2.97g	Sour-sweet	Light pink
Fleshman	4.08±0.15a	16.43±1.08a,b,c	0.15±0.00g	1.00±0.00c,d,e,f	129.99±5.68a	Sweet	Light pink
Haku-botan	2.66±0.03i	13.83±0.31e	2.97±0.29a	0.63±0.21f	5.43±0.38j	Sour	Cream
Salavatski	3.22±0.20g,h	16.40±0.20a,b,c	1.57±0.03b,c	1.57±0.21b	17.18±0.79g,h	Sour-sweet	Light pink
Cranberry	3.25±0.24g,h	15.63±0.49a,b,c,d	0.82±0.08d,e	1.33±0.15b,c,d	22.29±3.94g	Sour-sweet	Light and dark pink
Pink	3.77±0.20a,b,c,d	14.40±0.78d,e	0.26±0.00g,f	1.40±0.10b,c,d	74.44±3.24e	Sweet	Light pink

[A] Values are the averages of triplicates ± standard deviation. Values with the same letter for each cultivar in the same column are not significantly different at $p \leq 0.05$

Table 4.1c Characteristics of pomegranate juice extracted with mechanical press[A]

Cultivar	pH	TSS (°Brix)	TA (%citric acid)	Formol no (mL 0.1N NaOH/100 mL)	Maturity index (TSS:TA)	Type	Aril color
Kaj-acik-anor	3.23±0.14c,d,e	15.83±0.51a,b,c	1.02±0.27c,d,e,f	1.03±0.32a,b,c,d,e	15.31±2.03f,g	Sour-sweet	Dark pink
Rose	3.62±0.09b,c	14.97±1.32a,b,c,d	0.30±0.05g	0.93±0.06c,d,e	29.84±1.39e	Sour-sweet	Light and dark pink
Don Sumner South Tree	3.75±0.05a,b	14.37±0.38b,c,d	0.16±0.01g	0.77±0.15c,d,e	69.14±3.39c	Sweet	Light cream
Don Sumner North Tree	3.43±0.08b,c	14.23±0.38c,d	0.15±0.00g	0.70±0.10d,e	61.50±8.57d	Sweet	Light pink
King	3.72±0.06a,b	15.03±0.38a,b,c,d	0.13±0.00g	0.83±0.23c,d,e	95.33±3.15b	Sweet	Light cream
Crab	3.06±0.03d,e	15.63±1.29a,b	1.08±0.38c,d	0.90±0.17c,d,e	14.72±5.63f,g	Sour	Dark pink
Thompson	3.72±0.15a,b	14.63±0.32b,c,d	0.14±0.01g	1.07±0.15a,b,c,d	102.16±7.45a,b	Sweet	Light pink
Entek Habi Saveh	2.92±0.24e	15.63±0.47a,b	1.29±0.09b,c	1.43±0.12a	8.67±3.19g,h	Sour	Light and dark pink
Afganski	2.93±0.13e	14.37±0.47b,c,d	1.44±0.15b	0.93±0.31c,d,e	9.85±2.86g,h	Sour	Light and dark pink
Nikitski ranni	3.53±0.10b,c	15.17±0.25a,b,c	0.69±0.09f	1.07±0.06a,b,c,d	21.10±4.50f	Sour-sweet	Light pink
Fleshman	4.03±0.02a	16.27±0.31a	0.13±0.00g	1.00±0.00b,c,d,e	105.32±3.97a	Sweet	Light pink
Haku-botan	2.50±0.23f	13.80±0.78d	2.56±0.21a	0.60±0.10e	4.67±0.27h	Sour	Cream
Salavatski	2.86±0.01e	15.37±0.65a,b,c	0.96±0.03d,e	1.37±0.42a,b	9.82±0.62g,h	Sour	Light pink
Cranberry	3.10±0.11d,e	14.53±0.32b,c,d	0.67±0.12f	0.87±0.15c,d,e	19.07±1.46f	Sour-sweet	Light and dark pink
Pink	3.48±0.44b,c	14.27±0.40c,d	0.19±0.01g	0.90±0.17c,d,e	56.25±3.05d	Sweet	Light pink

[A] Values are the averages of triplicates ± standard deviation. Values with the same letter for each cultivar in the same column are not significantly different at $p \leq 0.05$

Table 4.2a Individual anthocyanins in blender extracted juice determined by RP-HPLC (mg/100 g FW)[A]

Cultivar	Blender						
	Cya3	Cya3,5	Dp3	Dp3,5	Pg3	Pg3,5	Total anthocyanins
Kaj-acik-anor	0.7±0.2a	1.16±0.14a	4.52±0.08a,b	0.29±0.03d	0.04±0.01a,b	0.15±0.02a,b	6.86±0.26a,b
Rose	0.08±0.00f	0.75±0.04c	0.00±0.00e	0.02±0.00h	0.00±0.00g,f	0.18±0.01a	1.03±0.05f,g,h
Don Sumner South Tree	0.14±0.00e	0.12±0.02h	0.00±0.00e	0.18±0.02e,f	0.00±0.00e,f,g	0.01±0.00f	0.45±0.02f
Don Sumner North Tree	0.16±0.01c,d,e	0.27±0.04g	2.08±0.11b,c	0.68±0.03a,b	0.00±0.00d,e,f,g	0.02±0.00e,f	3.21±0.37c,d,e
King	0.18±0.02c,d	0.38±0.06f,g	1.64±0.10c,d	0.45±0.06c	0.00±0.00d,e,f,g	0.04±0.01d,e	2.69±0.28d,e,f
Crab	0.15±0.02d,e	1.09±0.12b	0.00±0.00e	0.55±0.05b	0.00±0.00d	0.17±0.03a	1.96±0.27c,d,e
Thompson	0.17±0.00c,d,e	0.40±0.04e,f,g	0.73±0.19d,e	0.57±0.06a,b	0.00±0.00e,f,g	0.04±0.01d	1.91±0.29e,f
Entek Habi Saveh	0.17±0.02c,d,e	0.58±0.07d	0.00±0.00e	0.66±0.08a	0.00±0.00d,e	0.06±0.01c,d	1.47±0.08e,f
Afganski	0.20±0.02c	0.34±0.05g	2.03±0.06b	0.21±0.07d,e,f	0.01±0.00c	0.04±0.01d,e	2.83±0.48c,d,e
Nikitski ranni	0.16±0.01d,e	0.40±0.06e,f,g	0.00±0.00e	0.06±0.00g,h	0.00±0.00e,f,g	0.04±0.01d,e	0.66±0.08g,h
Fleshman	0.15±0.00d,e	0.49±0.02d,e,f	0.00±0.00e	0.27±0.01d,e	0.00±0.00e,f,g	0.07±0.00c	0.98±0.03f,g,h
Haku-botan	0.09±0.00f	0.00±0.00h	0.02±0.01e	0.01±0.00h	0.00±0.00d,e	0.00±0.00c,d	0.12±0.01h
Salavatski	0.19±0.02c,d	0.53±0.07d,e	0.00±0.00e	0.31±0.04d	0.00±0.00d,e,f	0.06±0.01c,d	1.09±0.09e,f
Cranberry	0.17±0.02c,d,e	0.99±0.16b	0.00±0.00e	0.14±0.02g,f	0.00±0.00d,e,f,g	0.12±0.02b	1.42±0.22f,g
Pink	0.20±0.05c	0.73±0.10c	2.17±0.06b	0.60±0.09a,b	0.00±0.00d,e,f	0.04±0.01d,e	3.74±0.19b,c,d

[A]Values are the averages of triplicates ± standard deviation. Values with the same letter for each cultivar in the same column are not significantly different at $p \leq 0.05$

Table 4.2b Individual anthocyanins in mechanical press extracted juice determined by RP-HPLC (mg/100 g FW)[A]

Cultivar	Mechanical press						
	Cya3	Cya3,5	Dp3	Dp3,5	Pg3	Pg3,5	Total anthocyanins
Kaj-acik-anor	0.39±0.14a,b	0.94±0.13a	3.33±0.05a	0.23±0.02d,e	0.02±0.01a	0.11±0.01b	5.02±0.28a
Rose	0.07±0.00h	0.64±0.08c	0.00±0.00e	0.01±0.00h	0.00±0.00g,f	0.11±0.01b	0.83±0.09e,f
Don Sumner South Tree	0.14±0.01e,f,g	0.08±0.01f	0.44±0.13d,e	0.03±0.00h	0.00±0.00e,f,g	0.00±0.00g	0.69±0.10f,g,h
Don Sumner North Tree	0.16±0.01d,e,f	0.26±0.05e	2.04±0.18b	0.52±0.07a	0.00±0.00e,f,g	0.02±0.00e,f	3.00±0.54b
King	0.13±0.01g,f	0.27±0.04e	1.27±0.20c,d	0.36±0.05c	0.00±0.00d,e	0.03±0.00e	2.48±0.12c,d
Crab	0.13±0.02g	1.04±0.14a	0.88±0.09d,e	0.28±0.04d	0.00±0.00c,d	0.15±0.01a	1.62±0.24d,e
Thompson	0.13±0.00g	0.22±0.03e	0.00±0.00e	0.11±0.01g,f	0.00±0.00f,g	0.03±0.01e	0.49±0.05f
Entek Habi Saveh	0.16±0.01c,d,e	0.52±0.06d	2.36±0.11b	0.05±0.00g,h	0.00±0.00d,e,f	0.06±0.01d	3.15±0.35b,c
Afganski	0.19±0.03c	0.27±0.04e	1.63±0.17b,c,d	0.13±0.02f	0.00±0.00c	0.03±0.00e	2.25±0.21c,d
Nikitski ranni	0.14±0.00g,f	0.29±0.04e	0.00±0.00e	0.05±0.01g,h	0.00±0.00f,g	0.03±0.01e	0.51±0.06f
Fleshman	0.14±0.00e,f,g	0.21±0.04e	0.00±0.00e	0.05±0.00g,h	0.00±0.00e,f,g	0.03±0.01e	0.43±0.05f
Haku-botan	0.00±0.00i	0.00±0.00f	0.00±0.00e	0.01±0.00h	0.00±0.00g	0.00±0.00g	0.01±0.00f
Salavatski	0.16±0.01c,d,e	0.32±0.04e	2.15±0.10b,c	0.03±0.00h	0.00±0.00e,f,g	0.02±0.00e	2.68±0.75c,d,e
Cranberry	0.15±0.00e,f,g	0.49±0.09d	0.00±0.00e	0.03±0.00h	0.00±0.00g	0.07±0.01d	0.74±0.12f
Pink	0.18±0.02c,d	0.33±0.05e	1.40±0.17b,c,d	0.46±0.06b	0.00±0.00e,f,g	0.01±0.00f,g	2.38±0.10b

[A]Values are the averages of triplicates ± standard deviation. Values with the same letter for each cultivar in the same column are not significantly different at $p \leq 0.05$

Cya3: cyanidin 3-glucoside, Cy3,5: cyanidin 3,5-diglucoside, Dp3: delphinidin 3-glucoside, Dp3,5: delphinidin 3,5-diglucoside, Pg3: pelargonidin 3-glucoside, Pg3,5: pelargonidin 3,5-diglucoside

Table 4.3a Color determination of aril juices extracted using blender[A]

Cultivar	Blender					
	L*	a*	b*	C*	h	Color index
Kaj-acik-anor	55.6±0.11j	47.36±0.06a	26.68±0.04a	54.36±0.07a	29.40±0.02m	1.37±0.00e
Rose	53.73±0.25k	35.87±0.16d	22.94±0.13d	42.58±0.20c	32.50±0.23k	1.53±0.02a
Don Sumner South Tree	78.94±0.01d	3.67±0.24l	22.98±0.35l	23.27±0.38g	83.04±0.02b	0.95±0.01j
Don Sumner North Tree	81.32±0.61b	7.27±0.12j	9.65±0.73j	11.93±0.23j	63.71±0.20f	0.80±0.00l
King	76.81±0.00e	8.72±0.01i	17.13±0.03i	17.75±0.04i	74.80±0.04e	1.11±0.00g
Crab	59.08±0.52i	40.86±0.00b	24.19±0.02b	47.48±0.01b	30.71±0.15l	1.40±0.00d
Thompson	79.62±0.01c,d	5.15±0.01k	19.16±0.26k	19.75±0.29h	75.93±0.17d	1.05±0.00h
Entek Habi Saveh	72.84±0.04f	39.05±0.01c	20.39±0.03c	44.06±0.02c	27.57±0.03n	1.52±0.00b
Afganski	66.00±1.28h	34.08±0.86e	19.92±0.75e	40.14±0.11d	35.25±0.07j	1.36±0.00e
Nikitski ranni	70.62±1.10g	18.96±0.03h	18.65±0.88h	25.41±0.74f	48.06±0.18h	1.37±0.00e
Fleshman	79.07±0.04c,d	8.25±1.56i,j	22.56±0.08i,j	24.44±0.25g	78.62±0.02c	0.98±0.02i
Haku-botan	83.00±0.08a	-0.30±0.08m	21.75±0.51m	21.75±0.51g	94.03±0.05a	0.82±0.00k
Salavatski	64.57±0.81h	30.82±0.80f	16.09±0.70f	35.44±0.12e	33.87±0.20k	1.46±0.00c
Cranberry	65.19±2.26h	30.73±0.40f	18.43±0.41f	34.98±0.53e	39.31±0.28i	1.40±0.00d
Pink	80.57±0.26b,c	18.72±1.25g	17.85±0.90g	25.07±0.32f	55.88±0.18g	1.17±0.00f

[A]Values are the averages of triplicates ± standard deviation. Values with the same letter for each cultivar in the same column are not significantly different at $p \leq 0.05$

Table 4.3b Color determination of aril juice extracted using mechanical press[A]

Cultivar	Mechanical press					
	L*	a*	b*	C*	h	Color index
Kaj-acik-anor	50.36±0.07j	45.17±0.11a	22.02±0.10a	50.25±0.13a	25.98±0.07l	1.53±0.00f
Rose	52.78±0.56i	35.39±0.29c	19.61±0.16c	40.46±0.32c	29.00±0.03j	1.62±0.00c
Don Sumner South Tree	71.89±0.59c,d	2.21±0.01k	18.12±0.01k	18.25±0.01h	80.93±0.46b	1.09±0.00m
Don Sumner North Tree	80.59±0.18a	4.76±0.32i	9.46±0.19i	10.76±0.81l	52.46±0.11f	1.40±0.00i
King	76.48±0.05b	4.65±0.02i	14.94±0.01e	17.30±0.01h	59.75±0.04e	1.28±0.00j
Crab	55.85±0.02h	37.93±0.42b	22.54±0.38a	44.13±0.55b	30.62±0.03i	1.49±0.00g
Thompson	75.51±0.24b	4.80±0.13i	14.69±0.01e	15.57±0.01i	70.67±0.02c	1.20±0.00l
Entek Habi Saveh	58.27±0.01g	21.32±0.09f	10.84±0.05g	23.92±0.10g	26.97±0.06k	1.86±0.00a
Afganski	63.51±0.93f	27.15±1.24d	19.19±0.93b	32.25±0.26d	30.31±0.32i	1.56±0.01e
Nikitski ranni	70.29±0.09d	16.77±0.89g	16.09±0.04d	24.87±0.05g	40.33±0.02h	1.46±0.00h
Fleshman	72.46±2.73c	3.39±0.00j	16.83±0.01d	17.17±0.01h	67.90±0.02d	1.25±0.00k
Haku-botan	79.62±0.43a	-0.96±0.01l	13.62±0.10f	13.65±0.10j	90.78±0.23a	0.95±0.00n
Salavatski	70.68±0.58d	21.97±0.54f	14.75±0.47e	26.46±0.71f	27.55±0.43k	1.57±0.01d
Cranberry	64.00±0.48f	23.33±1.81e	16.71±0.36b	29.14±0.77e	28.54±0.22j	1.62±0.00c
Pink	66.14±1.70e	16.85±0.15h	10.11±0.28g,h	12.22±0.32k	43.66±0.46g	1.74±0.01b

[A]Values are the averages of triplicates ± standard deviation. Values with the same letter for each cultivar in the same column are not significantly different at $p \leq 0.05$.

Table 4.4a Correlations for the various chemical analyses[a]

Variables	Blender		Mechanical press	
	Light juice[b]	Dark juice[c]	Light juice[b]	Dark juice[c]
TPP vs FRAP	0.73*	0.78	0.88*	0.43
TPP vs TEAC	0.47	0.82*	0.42	0.73
TPP vs ORAC	0.85*	0.65	0.82*	0.68
TMA vs TPP	-0.47	0.17	0.08	-0.63
TMA vs Total anthocyanins[d]	0.14	0.77*	0.71*	0.54
TMA vs FRAP	-0.27	0.44	0.08	0.01
TMA vs TEAC	-0.08	-0.23	0.32	-0.38
TMA vs ORAC	-0.23	-0.37	0.13	-0.53
pH vs TA	-0.94*	-0.54	-0.89*	-0.87*
pH vs TSS	0.55	0.77	0.48	0.02
TMA vs a*	-0.31	0.79	0.45	0.91*
TMA vs b*	0.03	0.83*	-0.52	0.66
TMA vs C*	-0.18	0.78	-0.05	0.90*
Maturity index vs TA	-0.82*	0.07	-0.76*	-0.96*
Maturity index vs TSS	0.40	0.31	0.33	-0.18
FRAP vs TEAC	0.16	0.73	0.47	0.78
FRAP vs ORAC	0.50	0.63	0.83*	0.83*
TEAC vs ORAC	0.78*	0.91*	0.73*	0.84*

[a]The r value of correlation is given and its significance ($p \leq 0.05$) identified by an asterisk

[b]Light color juice. Cultivars-King, Pink, Thompson, Fleshman, Salavatski, Nikitski ranni, Don Sumner South Tree, Don Sumner North Tree, Haku-botan

[c]Dark color juice. Cultivars-Cranberry, Crab, Kaj-acik-anor, Afganski, Entek Habi Saveh, Rose

[d]Obtained as a sum of individual concentrations of anthocyanins determined by RP-HPLC at 520 nm

TPP-total polyphenols; FRAP-ferric reducing antioxidant power; TEAC-trolox equivalent antioxidant capacity; ORAC-oxygen radical absorbance capacity; TMA-total monomeric anthocyanins; TA-titratable acidity; TSS-total soluble solids

Table 4.4b Values obtained for various analyses using the two extraction methods[A]

Analysis	Blender	Mechanical press
Yield (% FW)	42.04±3.74a	38.06±3.21b
TPP (mg GAE/100g FW)	64.94±3.25a	54.78±2.31b
FRAP (µM TE/g FW)	31.76±1.63a	27.21±1.81b
TEAC (µM TE/g FW)	6.54±0.25a	5.83±0.43b
ORAC (µM TE/g FW)	1290.07±3.11a	1158.38±3.19a
Total monomeric anthocyanins (mg cyanidin 3-glucoside/100g FW)[1]	13.33±1.49a	8.07±1.47b
pH	3.49±0.14a	3.33±0.13a
Total soluble solids (°Brix)	15.59±0.51a	14.94±0.55b
Titratable acidity (%citric acid)	0.91±0.17a	0.73±0.09a
Formol number	1.16±0.19a	0.96±0.17b
Maturity index	53.13±3.16a	41.52±3.44a
Color index	1.22±0.00b	1.44±0.00a
Total anthocyanins (mg/100g FW)[2]	2.03±0.18a	1.75±0.20a

[A]Values are the averages of triplicates ± standard deviation. Values with the same letter for each analysis in each row are not significantly different at $p \leq 0.05$

[1]Determined by pH differential method using spectrophotometer at 520 and 720 nm

[2]Obtained as a sum of individual anthocyanin concentrations determined by RP-HPLC at 520 nm

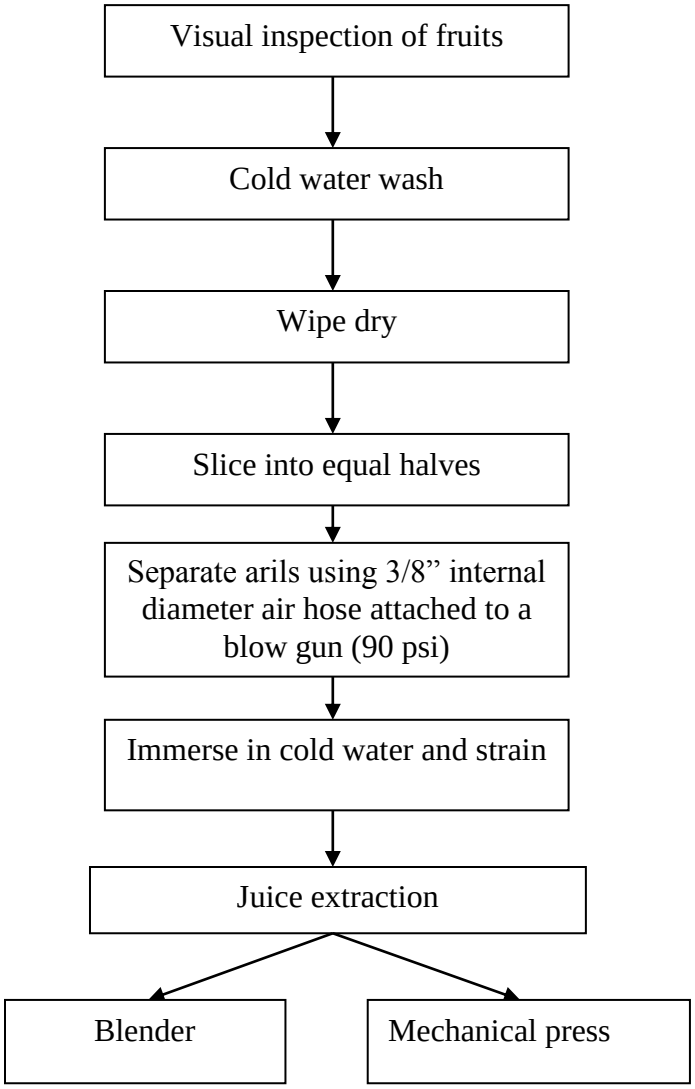
Visual inspection of fruits
Cold water wash
Wipe dry
Slice into equal halves
Separate arils using 3/8” internal diameter air hose attached to a blow gun (90 psi)
Immerse in cold water and strain
Juice extraction
Blender
Mechanical press

Figure 4.1a

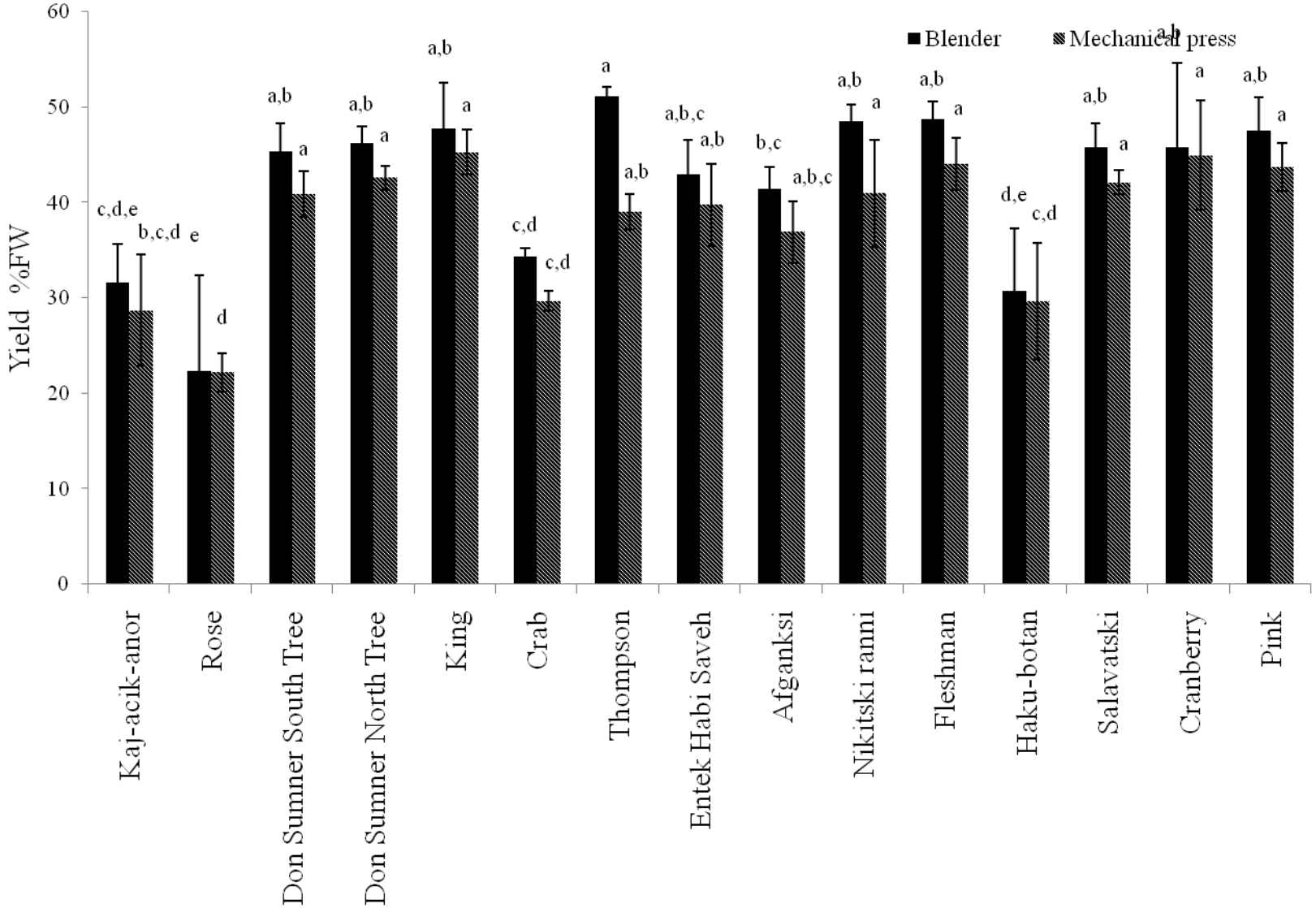
Yield %FW
60
50
40
30
20
10
0
Blender
Mechanical press
c,d,e
b,c,d
e
d
a,b
a
a,b
a
a,b
a
c,d
c,d
a
a,b
a,b,c
a,b
b,c
a,b,c
a,b
a
a,b
a
d,e
c,d
a,b
a
a,b
a
Kaj-acik-anor
Rose
Don Sumner South Tree
Don Sumner North Tree
King
Crab
Thompson
Entek Habi Saveh
Afganksi
Nikitski ranni
Fleshman
Haku-botan
Salavatski
Cranberry
Pink

Figure 4.1b

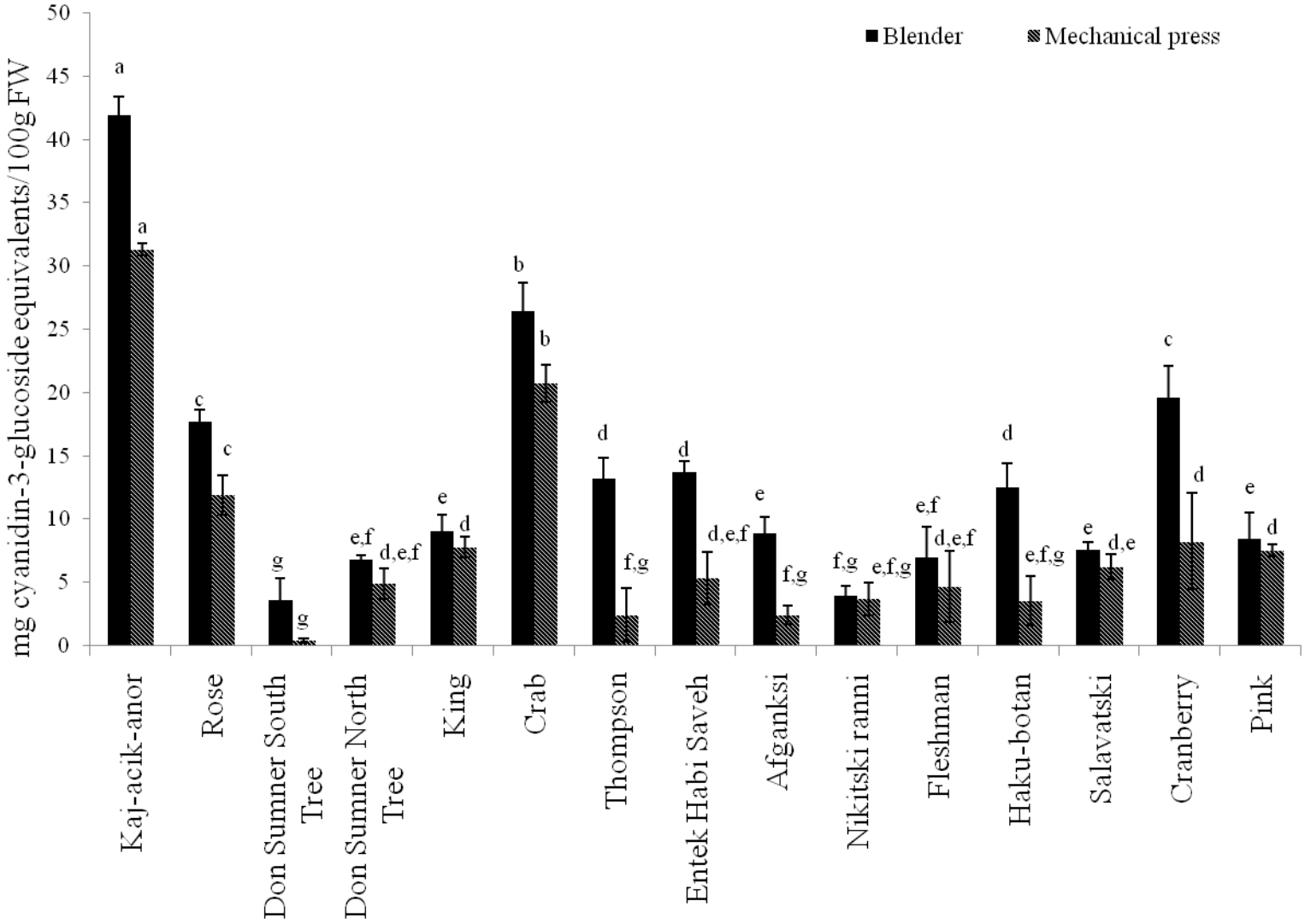
Blender
Mechanical press
mg cyanidin-3-glucoside equivalents/100g FW
50
45
40
35
30
25
20
15
10
5
0
a
a
c
c
g
g
e,f
d,e,f
e
d
b
b
d
f,g
d
d,e,f
e
f,g
f,g
e,f,g
e,f
d,e,f
d
e,f,g
e
d,e
c
d
e
d
Kaj-acik-anor
Rose
Don Sumner South Tree
Don Sumner North Tree
King
Crab
Thompson
Entek Habi Saveh
Afganksi
Nikitski ranni
Fleshman
Haku-botan
Salavatski
Cranberry
Pink

Figure 4.1c

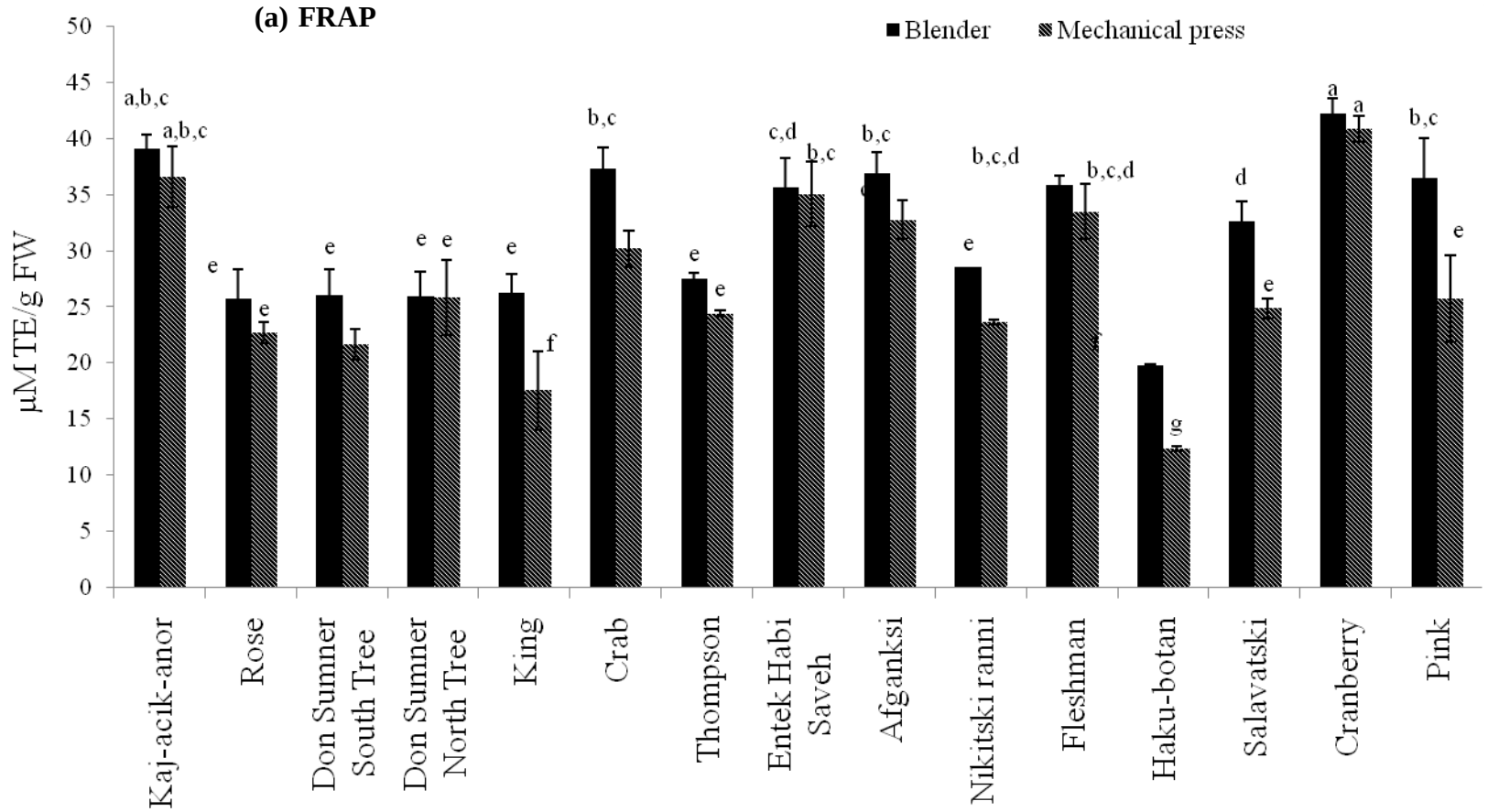
(a) FRAP
Blender
Mechanical press
µM TE/g FW
50
45
40
35
30
25
20
15
10
5
0
a,b,c
a,b,c
e
e
e
e
e
e
f
b,c
b,c
e
e
c,d
b,c
b,c
b,c,d
e
b,c,d
d
e
a
a
b,c
e
Kaj-acik-anor
Rose
Don Sumner South Tree
Don Sumner North Tree
King
Crab
Thompson
Entek Habi Saveh
Afganksi
Nikitski ranni
Fleshman
Haku-botan
Salavatski
Cranberry
Pink

Figure 4.2a

(b) TEAC

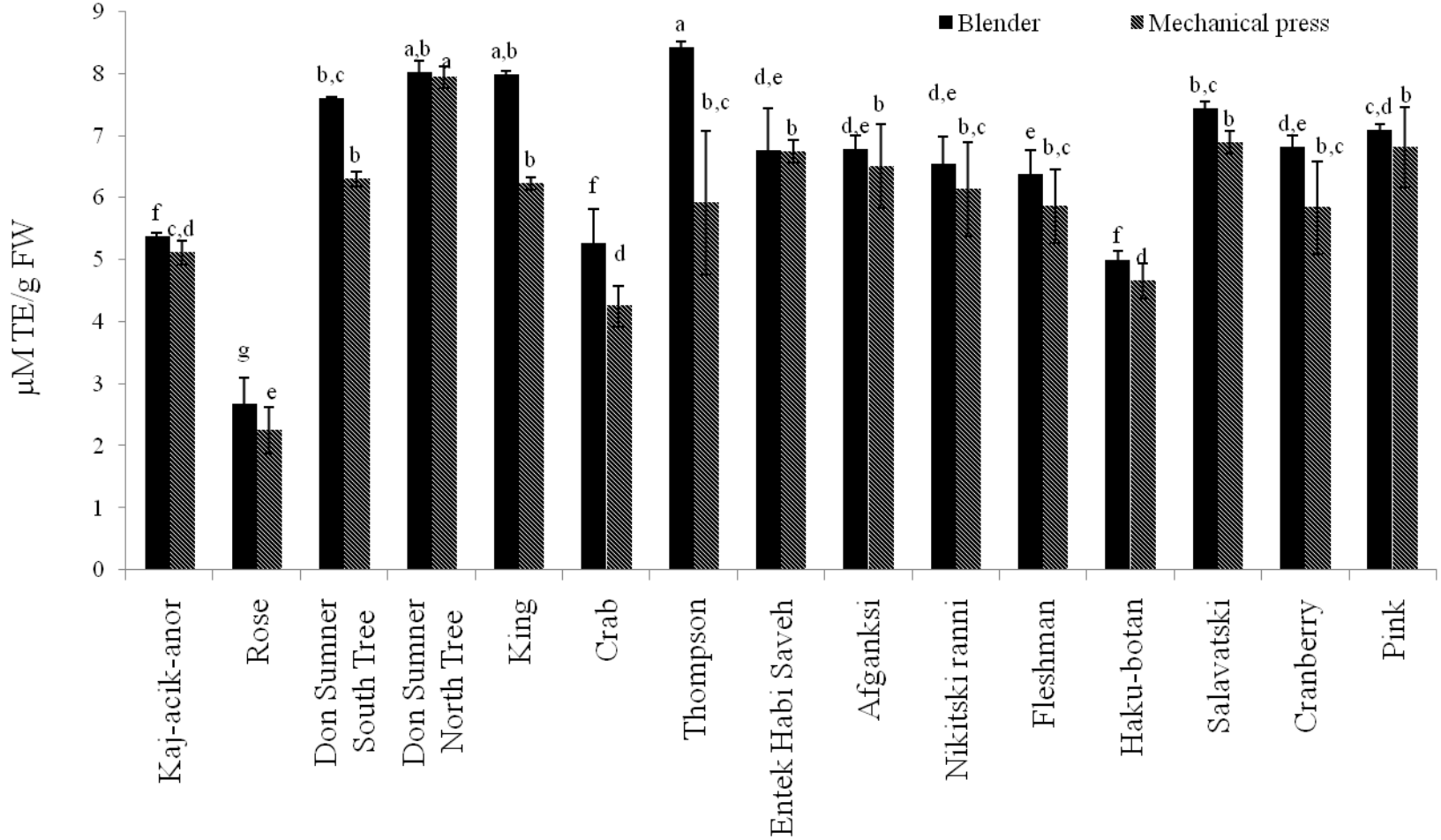

Figure 4.2b

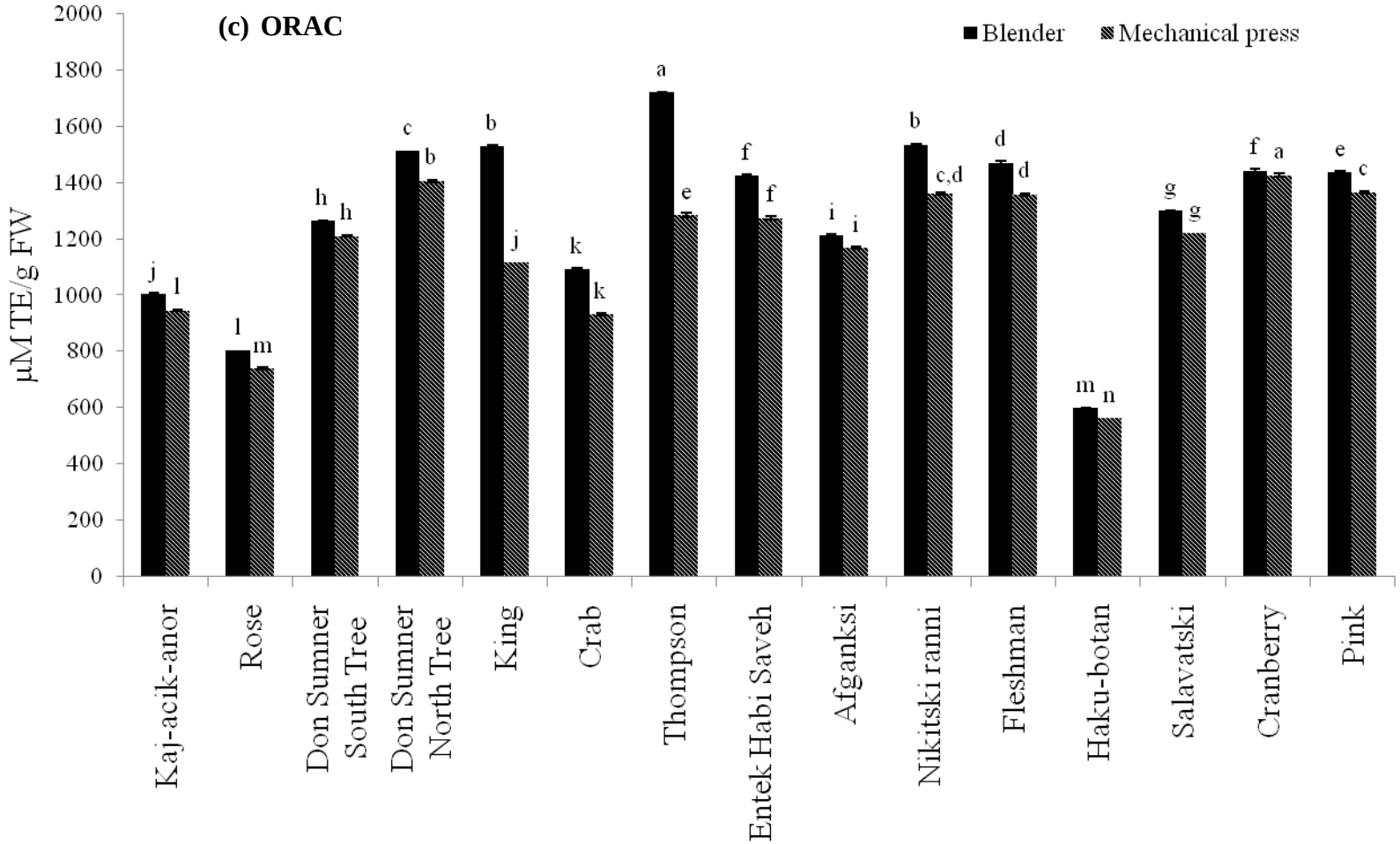
(c) ORAC
Blender
Mechanical press
µM TE/g FW
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Kaj-acik-anor
Rose
Don Sumner South Tree
Don Sumner North Tree
King
Crab
Thompson
Entek Habi Saveh
Afganksi
Nikitski ranni
Fleshman
Haku-botan
Salavatski
Cranberry
Pink

Figure 4.2c

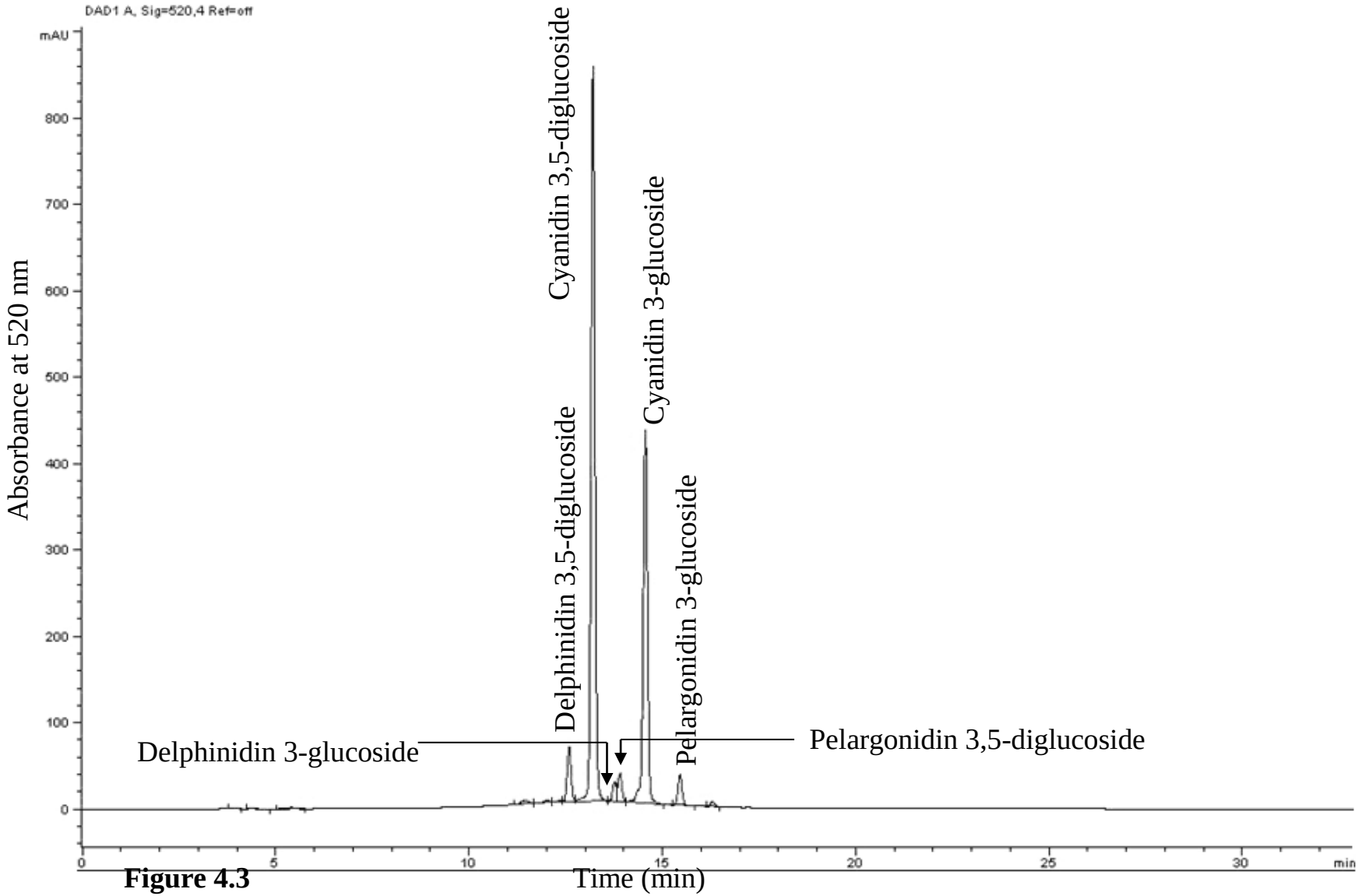
DAD1 A, Sig=520,4 Ref=off
mAU
800
700
600
500
400
300
200
100
0
Absorbance at 520 nm
Cyanidin 3,5-diglucoside
Cyanidin 3-glucoside
Delphinidin 3,5-diglucoside
Pelargonidin 3-glucoside
Delphinidin 3-glucoside
Pelargonidin 3,5-diglucoside
0
5
10
15
20
25
30
min
Time (min)

Figure 4.3

CHAPTER 5

TOTAL PHENOLICS AND ANTIOXIDANT CAPACITY OF POMEGRANATE ARIL JUICE EXTRACTS FROM 2009 AND 2010 HARVEST YEARS

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To be Submitted to Food Chemistry

Abstract

The potential health benefits of pomegranate (*Punica granatum* L.) can be attributed to their total phenolics content and antioxidant capacity. Arils from nine pomegranate cultivars harvested in 2009 and 2010 were juiced using two methods, blender and mechanical press. The yield was higher in 2010 (31.58; 25.32% FW), compared to 2009 (42.07; 38.52% FW), in blender and mechanical press, respectively. The total polyphenols was analyzed by Folin-Ciocalteu method, and the results were comparable between the two years. Total monomeric anthocyanins were analyzed based on pH-differential method. Cultivar Kaj-acik-anor had the highest value (36.56, 33.01; 41.97, 31.30 mg cyanidin-3-glucoside equivalents/100 g FW) for both years with blender and mechanical press, respectively. FRAP, TEAC, and ORAC were used to assess the antioxidant capacity of the aril juice. The antioxidant capacity of 2010 harvest was higher than the 2009 harvest. Significant correlations ($p \leq 0.05$) were observed between total polyphenols and FRAP. Seasonal variations may contribute to the differences in accumulation of phenolic compounds in pomegranates. Blender was an efficient method for aril juice extraction compared to mechanical press.

Keywords: Pomegranate (*Punica granatum* L.), aril juice, extraction methods, harvest year, yield, total polyphenols, antioxidant capacity, total monomeric anthocyanins.

Introduction

Pomegranate fruit (*Punica granatum* L.) has been extensively used in traditional medicine and is one of the oldest known edible fruits. Recent studies have shown the potential of pomegranate juice to act as chemopreventive, chemotherapeutic, anti-atherosclerotic, and anti-inflammatory agent. This has led to a growing demand for pomegranates and increased consumption of pomegranate juice (Faria, Monteiro, Mateus, Azevedo, & Calhau, 2007). Lansky & Newman (2007) reported that more than 1000 *Punica granatum* cultivars exist with origin in the Middle East. They are also grown in the Mediterranean region, China, India, California and Mexico. These regions have semi-arid mild- temperature to subtropical climates with hot summers and cool winters, which are ideal for pomegranate cultivation (Stover & Mercure, 2007).

The edible portion of the fruit is called arils and can be used for juice production and also for fresh consumption. They constitute 52% of total fruit (w/w) and primarily consist of 78% juice and 22% seeds (Kulkarni & Aradhya, 2005). The antioxidant capacity of pomegranate juice is due to the presence of polyphenols such as anthocyanins, phenolic acids, hydrolyzable tannins, and ellagic acids. Commercial pomegranate juice has three times higher antioxidant capacity than green tea and red wine (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000). Seeram et al. (2008) reported that the antioxidant capacity of pomegranate juice is higher compared to other fruit juices and beverages.

The chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey have been studied (Özgen, Durgaç, Serçe, & Kaya,

2008). The changes in total anthocyanin and antioxidant capacity of pomegranate arils during fruit development were reported by Kulkarni & Aradhya (2005). The genetics of the fruit, maturity, environmental, agronomic and postharvest conditions, storage, and processing factors determine the composition and bioactive compounds present in pomegranate juice (Miguel, Dandlen, Antunes, Neves, & Martins, 2004; Poyrazoğlu, Gökmen, & Artík, 2002).

In Georgia, pomegranate cultivation is in early stages. The aim of this present work is to compare pomegranate aril juice based on yield, total phenolics content, antioxidant activity, and total anthocyanin levels, from nine Georgia-grown pomegranate cultivars for two harvest years (2009 & 2010). The juice was extracted with blender or mechanical press. Therefore, comparison between both methods was also performed for the various chemical analyses.

Materials and methods

Plant material

Nine pomegranate (*P. granatum*, Punicaceae) cultivars grown in Georgia were used in this study. Don Sumner South Tree, Don Sumner North Tree, Haku-botan, Salavatski, Kaj-acik-anor, Nikitski ranni, Afganski, Entek Habi Saveh, and Cranberry were obtained from the University of Georgia Ponder farm, located near Tifton, GA. The trees at the Ponder Farm were planted in a loamy-sand soil (sand, 86%; silt, 7%; and clay, 7%) from 1990 to 1993. Orchard management was minimal until 2008, with no supplemental fertilizer or irrigation applied. Pruning was performed at irregular intervals since the initial planting. The average maximum and minimum temperatures for 2009 was 76.02 °F and 56.1 °F, and for 2010, 76.03 °F and 54.06 °F, respectively. The total

rainfall for 2009 and 2010 was 56.79 and 43.28, respectively. The average total rainfall for 2009 was 0.15 and for 2010, 0.11 inches. Fruits were harvested at maturity for 2009 and 2010 as estimated based on soluble sugar content, color, and total acidity. They were then transported to the University of Georgia Vidalia Onion Research Laboratory, where fruits were cooled to 7 °C prior to subsequent analysis.

Chemicals

Folin-Ciocalteu reagent, 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and potassium persulfate were purchased from Sigma Chemical Co. (St. Louis, MO). 2, 4, 6-Tripyridyl-s-triazine (TPTZ) and 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Acros Organics (Morris Plains, NJ) and $FeCl_3.6H_2O$ from Fluka (Milwaukee, WI). Other solvents and chemicals were purchased from Sigma Chemical Co., J. T. Baker Chemical Co. (Phillipsburg, NJ), and/ or Fischer Scientific (Norcross, GA).

Sample preparation

The fruits were washed with water and wiped completely dry. Fruits from each cultivar were then divided into equal portions for juice extraction with either an Oster® blender (Oster, Fort Lauderdale, FL) or a hand operated juice extractor/mechanical press (Strite-Anderson Mfg. Co., Minneapolis, MN). The juice was obtained by pressurization of the arils. In the blender, the white membrane and the arils were juiced while in the juice extractor, it was only the aril juice (Fig. 5.1a). All sample preparation was done under dark conditions. The juice was flushed with nitrogen and stored at –80 °C until further analysis. All extractions were performed in triplicate.

Total polyphenols (TPP)

Total polyphenols were determined according to the Folin-Ciocalteu reagent method (Singleton & Rossi, 1965). To each 50 μL of extracted juice sample, 0.5 mL of Folin-Ciocalteu reagent and 1.5 mL of 7.5% sodium carbonate solution were added. The samples were then mixed well and allowed to stand for 30 min in the dark at room temperature. Absorption at 765 nm was read using a Shimadzu 300 UV-vis spectrophotometer (Shimadzu UV-1601, Norcross, GA). Quantification was based on the standard curve generated with 1-15 mg/L of gallic acid, and average results from triplicate determinations reported as mg GAE/100 g FW.

Antioxidant capacity

Ferric reducing antioxidant capacity (FRAP) assay

The FRAP assay was performed according to the method of Benzie and Strain (1996) with minor modifications. Stock solutions of 300 mM acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine solution in 40 mM HCl), and 20 mM $FeCl_3.6H_2O$ were prepared. The FRAP reagent was prepared by mixing the stock solutions in 10:1:1 ratio and maintained at 37 °C and pH 3.6. Then, 10 μL of the sample and 300 μL of FRAP reagent were added in a 96-well microplate (Tsao, Yang, Xie, Sockovie, & Khanizadeh, 2005) and incubated at room temperature for 4 min. The absorbance was measured at 595 nm using a microplate reader (BioRad 680 XR, Hercules, CA). Trolox calibration solutions (100, 200, 400, 500 and 750 μM) were used to generate the standard curve and the results were expressed as μM Trolox equivalents (TE)/g FW. All assays were done in triplicate and averages were reported.

Trolox equivalent antioxidant capacity (TEAC) assay

The assay was performed based on the method of Lee, Kim, Kim, Lee, & Lee (2003) with slight modifications. Briefly, 7 mM ABTS solution and 2.45 mM potassium persulfate solution were mixed and kept in the dark at room temperature for 12-16 h. The $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. To each 10 µL aliquot of Trolox standard or sample, 200 µL of diluted $ABTS^{\cdot+}$ was added , and the absorbance was read for 6 min at 734 nm using a microplate reader (BioRad 680 XR, Hercules, CA). The percent inhibition of absorbance was calculated and plotted as a function of Trolox concentration. TEAC values of samples were calculated from the standard curve and reported as µM TE/g FW from the average of triplicate determinations.

Oxygen radical scavenging capacity (ORAC) assay

Briefly, 25 µL of Trolox standard or pomegranate juice in 75 mM potassium phosphate buffer, pH 7.4 (working buffer), was added in triplicate wells to a 96-well, black, clear bottom microplate. 150 µL of 0.96 µM fluorescein in working buffer was added to each well and incubated at 37 °C for 20 min, with intermittent shaking. After incubation, 25 µL of freshly prepared 119 mM 2,2'-azobis(2-amidinopropane) dihydrochloride (ABAP) in working buffer was added to the wells using a 12-channel pipetter. The microplate was immediately inserted into a Synergy™ HT plate reader (Biotek Instruments, Winooski, VT) at 37 °C. The decay of fluorescence at 528 nm was measured with excitation at 485 nm every minute for 60 min. Quantification was based on the standard curve generated with Trolox, and average results from triplicate analyses were reported as µM TE/g FW (Prior et al., 2003).

Total monomeric anthocyanins

The total anthocyanin content was estimated by the pH-differential method (AOAC method 2005.02) using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M) on a UV-vis spectrophotometer (Shimadzu UV-1601, Norcross, GA). Samples were diluted in pH 1.0 and pH 4.5 buffers and then measured at 520 and 700 nm. The absorbance was calculated as $A = (A_{520nm} - A_{700nm})_{pH\ 1.0} - (A_{520nm} - A_{700nm})_{pH\ 4.5.}$

The monomeric anthocyanin pigment concentration was calculated as cyanidin-3-glucoside. The monomeric anthocyanin pigment (mg/L) = A x MW x DF x 1000/(ε x 1), where A = absorbance, MW = molecular weight (449.2), DF = dilution factor, and ε = molar absorptivity (26900). All measurements were done in triplicate and averages were reported.

Statistical analysis

All samples were analyzed in triplicate and the results expressed as average ± standard deviation. All statistical analysis were conducted using one-way ANOVA. Duncan's multiple-range test was used to determine statistically significant differences of variables at $p \leq 0.05$ (SAS 8.2, SAS Inst., Inc., 1999). Correlation studies and their significance were performed using Pearson tests with Microsoft Excel software package (Microsoft Corp., Redmond, WA).

Results and discussion

Juice yield

Pomegranate juice processing industry is interested in cultivars with high juice yielding potential for commercial viability. For 2009 harvest season, cultivar Cranberry

had the highest juice yield with blender (41.26%), and mechanical press (36.31%) extractions. For 2010 harvest season, cultivar Nikitski ranni gave more juice yield with blender (48.56%), and cultivar Cranberry with mechanical press (44.98%) (Fig. 5.1b). The yield was calculated based on fresh weight (FW) of the fruits. The 2010 harvest values for juice yield was higher for the cultivars when compared to the 2009 yield values. Stover & Mercure (2007) suggested that climates which are semi-arid to subtropical with hot summers and cool winters are suitable for pomegranate cultivation. The 2010 year had dry spring and hot summer conditions with consistent thunderstorms. Thus, climate, temperature, and humidity may affect the number of arils and its juice levels (Borochov-Neori, Judeinstein, Tripler, Harari, Greenberg, Shomer et al., 2009). Schwartz, Tzulker, Glazer, Bar-Ya'akov, Wiesman, Tripler et al. (2009) and Borochov-Neori et al. (2009) also reported similar results and suggested that cultivar grown in Mediterranean-like climate had higher juice content in their arils.

Total phenolics content

The antioxidant capacity of pomegranate juice is high and it is known to be an effective scavenger of free radicals, mainly due to the presence of phenolic acids, flavonoids, and polyphenolic compounds (Aviram, Fuhrman, Rosenblat, Volkova, Kaplan, Hayek, et al., 2002; Kulkarni et al., 2005). The polyphenolic and antioxidant tests are based on REDOX reactions, as these molecules undergo REDOX reactions. This is due to the presence of phenolic hydroxyl groups which readily donate hydrogen to reducing agents. The Folin-Ciocalteau method was used for the determination of total polyphenolic compounds, because Folin-Ciocalteau is a REDOX reagent (Madrigal-Carballo, Rodriguez, Krueger, Dreher, Reed, 2009). The total polyphenols ranged from

34.07 - 85.84 mg GAE/100 g FW for 2009 harvest, and 27.25 - 84.94 mg GAE/100 g FW for 2010 harvest (Fig. 5.2). The average total phenolics levels for blender in 2009 and 2010 harvests were 63.41 and 64.79 mg GAE/100 g FW, respectively. The average total phenolics levels for mechanical press in 2009 and 2010 harvests were 49.55 and 58.01 mg GAE/100 g FW, respectively. This shows that the total phenolics levels for both years were comparable. However, significant differences were observed among different cultivars and similar results have been reported by Hernandez, Melgarejo, Tomas-Barberan & Artes (1999) and Poyrazoğlu et al. (2002).

Antioxidant capacity

Plants have developed a complex antioxidant system by producing increased levels of secondary metabolites like phenols (flavonoids, anthocyanins). This system inhibits the oxidative damage caused by reactive oxygen species (ROS). The ROS are inactivated by the phenolic compounds, which have antioxidant activities such as chelation of metals and free radical-scavenging capacity (Gil et al., 2000; Narayana, Reddy, Chaluvadi, Krishna, 2001). FRAP, TEAC, and ORAC were used to determine the antioxidant capacity of the juice. Cultivar Cranberry had the highest FRAP value in 2009 harvest with blender extraction and both methods for 2010 harvest (Fig. 5.3a). Cultivar Afganski had the highest FRAP value for mechanical press for 2009 harvest. The highest TEAC value for 2009 harvest was cultivar Cranberry, and for 2010 harvest, cultivar Don Sumner North Tree (Fig. 5.3b). FRAP and TEAC values for Georgia-grown pomegranate cultivars were previously reported by Pande & Akoh (2009). Cultivar Nikitski ranni had the highest ORAC value with blender extraction for 2009 and 2010 harvests (Fig. 5.3c). For mechanical press juice extraction method, cultivar Kaj-acik-anor had the highest

ORAC value for 2009 harvest and cultivar Cranberry for 2010 harvest. Based on the results between the two years, cultivar Cranberry had the highest antioxidant capacity. On an average, FRAP and ORAC value were higher in 2010 harvest season than 2009 harvest season (Table 5.4). This may be due to the cultivar difference, seasonal variations and maturity of the fruit at harvest. Table 5.1 shows the average maximum temperatures during the ripening season of the fruit. The year 2010 had higher temperatures compared to 2009. Gautier, Bénard, Reich, Buret, Bourgaud, Poëssel et al. (2008) reported that makor phenolic compounds in tomatoes significantly increased when fruit temperature increased from 27 to 32 °C to protect the fruit from oxidative stress induced by a temperature increase. Plants produce higher phenolic compounds when exposed to stress conditions like drought, wounding, metal toxicity, and lack of nutrients (Winkel-Shirley, 2001). This might account for higher antioxidant capacity in 2010 harvest. The year 2009 received more rain than average which may be the reason for lower antioxidant values. Similar results have been reported for apples and figs, respectively (Łata & Tomala, 2007; Veberic, Colaric, & Stampar, 2008). Heavy rainfall also produces fruits with low keeping quality and causes fruit splitting.

Total monomeric anthocyanins

Pomegranate juice is widely consumed for its antioxidant benefits. Consumers associate higher antioxidant benefits with its attractive red colored juice. It is important to quantify the total monomeric anthocyanin levels in different cultivars and determine their correlation with antioxidant capacity. Cultivar Kaj-acik-anor had significantly ($p \leq 0.05$) high total monomeric anthocyanin level for 2009 and 2010 harvest with blender and mechanical press extractions (Fig. 5.4). For 2009 harvest season, on average, blender

juice extraction resulted in 12.36 mg cyanidin-3-glucoside equivalents/100 g FW total anthocyanin levels, while for mechanical press it was 9.61 mg cyanidin-3-glucoside equivalents/100 g FW. For 2010 harvest season, the blender and mechanical press juice extraction had 13.14 and 7.33 mg cyanidin-3-glucoside equivalents/100 g FW, respectively. The main factor influencing aril color and total anthocyanin levels is temperature. Extremely hot temperatures result in fruits having low external and internal color and low anthocyanins levels compared to temperate climate environments. The harvest season for Georgia-grown pomegranate was in September for both years. It was reported that the anthocyanin levels were low in summer, slightly higher in autumn and highest in winter harvest pomegranate fruit arils (Borochov-Neori, Judeinstein, Harari, Bar-Ya'akov, Patil, Lurie et al., 2011).

Correlations

The total phenolics content was significantly correlated to antioxidant capacity measured by FRAP (Table 5.2) with blender for 2009 and 2010 harvest and mechanical press for 2010 harvest. The total phenolics content was also significantly correlated to ORAC value of juice extracted using mechanical press for 2010 harvest. This suggests that the polyphenols contributed significantly to the antioxidant capacity of the juice. Similar results were reported by Tzulker et al. (2007) and Schwartz et al. (2009). Negative correlation was observed between antioxidant capacity and total monomeric anthocyanin levels, suggesting that anthocyanins are not major contributors to antioxidant capacity. This result was comparable to Gil et al. (2000) and Borochov-Neori et al. (2009).

Significant correlations existed between FRAP and TEAC for blender in 2009 harvest season, and also between TEAC and ORAC for blender and mechanical press extracts for 2010 harvest season. This suggests that all the three methods are suitable for antioxidant determination of pomegranate juice. It is not appropriate to assess antioxidant capacity based on one assay, since antioxidants have a complex mechanism in a biological matrix and are based on several factors. Thus, the results must be based on different antioxidant tests to determine the various characteristics (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002).

Comparison between blender and mechanical press

For 2009 harvest, significant ($p \leq 0.05$) differences were observed for yield, total phenolic content, FRAP, and TEAC values, between the two methods of juice extraction (Table 5.4). The blender had a high total phenolics content and antioxidant capacity. The juice from the blender was a combination of white membrane, pith, arils, and seeds of the fruit. The juice from mechanical press was obtained by pressing only the arils. Studies suggest that the fruit membranes have the highest phenolic and antioxidant contents (Kulkarni & Aradhya, 2004; Rosenblat & Aviram, 2006). This may be the reason for the higher antioxidant capacity and total phenols for blender compared to mechanical press extraction. For 2010 harvest, significant differences were observed only for total monomeric anthocyanins. These results were not consistent with the 2009 results. This may be related to the color variation in the juice between the two years. Visually, the cultivars exhibited color differences in aril juice for both years.

There were significant ($p \leq 0.05$) differences observed for the two methods between 2009 and 2010 as shown in Table 5.3. The total phenolics content of juice

extracted with blender did not differ much between the two years of harvest. Also, total monomeric anthocyanins were not significantly different for the methods and year of harvest. These results show that the method of juice extraction significantly affects the chemical properties of the juice and they are not consistent in consecutive years of harvest.

Pomegranate aril juice was compared with other Georgia-grown crops and commercial fruit juices (Table 5.5). Pande & Akoh (2009) reported total phenolic content and TEAC values for lipophilic and hydrophilic fractions of pomegranate juice from six cultivars. The values obtained in our study was lower when compared with commercial pomegranate juice, POM Wonderful. This supports the findings of Gil et al. (2000) as they reported that the commercial pomegranate juices had higher phenolics content than juice produced in the laboratory using arils. The reason for this difference may be due to the presence of high levels of ellagic acid derivatives and punicalagin extracted from the rind by hydrostatic pressing of the whole fruit to release juice from arils during industrial processing.

Conclusion

The data reported here detailed the changes in yield, total phenolics levels, antioxidant capacity, and total monomeric anthocyanins between two years of harvest (2009 & 2010) for pomegranate aril juice extracted using two methods. Results show that the blender was a better and efficient method of extraction, compared to mechanical press. Year-to-year variations existed for the methods of extraction and cultivars, suggesting that the Phytochemical content is dependent on these factors, in addition to climate and environmental conditions. Cultivar Kaj-acik-anor had the highest total

monomeric anthocyanin for both years, while cultivar Cranberry had a high antioxidant capacity. Our results, based on variation in climate, may help the pomegranate growers in Georgia to enhance their breeding program and agricultural practices. It would also aid in the selection of appropriate cultivars for juice production to meet consumer demand for high quality fruits with good antioxidant properties.

Acknowledgement

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Figure captions

Fig. 5.1 (a) Scheme for juice extraction. (b) Yield based on FW. Values are the average of triplicates. Values with the same letter for each cultivar are not significantly different at $p \leq 0.05$

Fig. 5.2 Total polyphenols (TPP). Values are the average of triplicates. Values with the same letter for each cultivar are not significantly different at $p \leq 0.05$

Fig. 5.3 Antioxidant capacity by (a) FRAP, (b) TEAC, (c) ORAC assays. Values are the average of triplicates. Values with the same letter for each cultivar are not significantly different at $p \leq 0.05$

Fig. 5.4 Total monomeric anthocyanins. Values are the average of triplicates. Values with the same letter for each cultivar are not significantly different at $p \leq 0.05$

Table 5.1 Total rainfall, average rainfall and temperatures during the months April to September for the harvest years 2009 and 2010

	Year	April	May	June	July	August	September
Total rainfall (inches)	2009	10.01	3.9	1.56	5.3	7.5	0.98
	2010	3.68	5.56	5.97	3.14	5.99	1.6
Av. Rainfall (inches)	2009	0.33	0.13	0.05	0.17	0.24	0.03
	2010	0.12	0.18	0.19	0.10	0.19	0.05
Tmax (°F)	2009	75.14	82.07	91.75	89.50	88.34	85.62
	2010	78.82	85.82	92.00	93.00	91.53	89.23
Tmin (°F)	2009	53.63	64.75	71.43	70.18	70.88	67.78
	2010	53.94	65.5	71.43	72.56	74.10	66.32

Av: average; Tmax: maximum temperature; Tmin: minimum temperature

http://www.georgiaweather.net/

Table 5.2 Correlations for the various chemical analyses[a]

Tests	2009 harvest		2010 harvest	
	Blender	Mechanical press	Blender	Mechanical press
TPP vs FRAP	0.775*	0.442	0.738*	0.690*
TPP vs TEAC	0.550	0.036	0.258	0.499
TPP vs ORAC	-0.012	-0.355	0.646	0.771*
TPP vs TMA	-0.580	-0.513	0.283	0.031
TMA vs FRAP	-0.377	-0.108	0.549	0.468
TMA vs TEAC	-0.437	-0.225	-0.598	-0.397
TMA vs ORAC	0.237	0.416	-0.347	-0.231
FRAP vs TEAC	0.706*	0.600	0.015	0.129
FRAP vs ORAC	0.452	0.375	0.346	0.508
TEAC vs ORAC	0.109	0.024	0.773*	0.745*

[a]The r value of correlation is given and its significance ($p \leq 0.05$) identified by an asterisk

TPP-total polyphenols; FRAP-ferric reducing antioxidant power; TEAC-trolox equivalent antioxidant capacity; ORAC-oxygen radical absorbance capacity; TMA-total monomeric anthocyanins.

Table 5.3 Values obtained for various analyses using two extraction methods[A]

Tests	2009 harvest		2010 harvest	
	Blender	Mechanical press	Blender	Mechanical press
Yield (% FW)	31.58±1.77a	25.33±4.51b	42.08±3.78a	38.53±3.97a
TPP (mg GAE/100g FW)	63.41±1.01a	49.55±1.20b	64.78±3.52a	58.01±2.15a
Total monomeric anthocyanins (mg cyanidin 3-glucoside/100g FW)	12.36±2.19a	9.61±0.96a	13.15±1.29a	7.34±1.42b
FRAP (µM TE/g FW)	23.67±1.00a	15.94±0.92b	31.89±1.49a	28.21±1.61a
TEAC (µM TE/g FW)	9.03±0.62a	7.69±0.47b	6.70±0.23a	6.24±0.37a
ORAC (µM TE/g FW)	609.45±5.55a	592.18±7.15a	1255.12±2.81a	1175.46±3.09a

[A]Values are the averages of triplicates ± standard deviation. Values with the same letter for each analyses in each row for each harvest are not significantly different at $p \leq 0.05$.

Table 5.4 Values obtained for various analyses using two extraction methods for 2009 and 2010[A]

Tests	Blender		Mechanical press	
	2009 harvest	2010 harvest	2009 harvest	2010 harvest
Yield (% FW)	31.58±1.77b	42.08±3.78a	25.33±4.51b	38.53±3.97a
TPP (mg GAE/100g FW)	63.41±1.01a	64.78±3.52a	49.55±1.20b	58.01±2.15a
Total monomeric anthocyanins (mg cyanidin 3-glucoside/100g FW)	12.36±2.19a	13.15±1.29a	9.61±0.96a	7.34±1.42a
FRAP (µM TE/g FW)	23.67±1.00b	31.89±1.49a	15.94±0.92b	28.21±1.61a
TEAC (µM TE/g FW)	9.03±0.62a	6.70±0.23b	7.69±0.47a	6.24±0.37b
ORAC (µM TE/g FW)	609.45±5.55b	1255.12±2.81a	592.18±7.15b	1175.46±3.09a

[A]Values are the averages of triplicates ± standard deviation. Values with the same letter for each analyses in each row for each method are not significantly different at $p \leq 0.05$.

Table 5.5 Comparison of Pomegranate with other Georgia-grown crops and other fruits and fruit juices

Fruit	Total polyphenols (mg GAE/100 g FW)	TEAC (µM TE/g FW)	Reference
Pomegranate aril juice	64.09±2.27[A]	7.87±0.43[A]	
Pomegranate pulp	164.4±6.4[B]	26.5±2.1[B]	Pande & Akoh, 2009
Other Georgia-grown crops			
Rabbiteye blueberries	556.1±216.9	27.6±5.3	Sellappan, Akoh, & Krewer, 2002)
Southern highbush blueberries	399.3±149.1	14.8±8.2	Sellappan et al., 2002
Blackberries	486.5±97.1	20.4±3.3	Sellappan et al., 2002
Muscadine-purple (whole fruit)	247.7±100.5	17.6±7.1	Pastrana-Bonilla, Akoh, Sellappan, & Krewer, 2003)
Apple juice[C]		4.3±0.3[I]	Seeram, Aviram, Zhang, Henning, Feng, Dreher et al., 2008
Red wine[D]		19.8±0.4[I]	Seeram et al., 2008
Pomegranate juice[E]		41.6±1.8[I]	Seeram et al., 2008
Acai juice[F]		12.8±0.4[I]	Seeram et al., 2008
Blueberry juice[G]		14.7±0.5[I]	Seeram et al., 2008
Cranberry juice[H]		9.6±0.4[I]	Seeram et al., 2008

[A]Average±standard deviation of nine cultivars in 2009 and 2010 extracted using blender, [B]Sum of hydrophilic and lipophilic fractions [C]Dole apple juice (Pepsico, NY),[D]Merlot Beringer (Beringer Vineyards, Napa, CA), [E]POM Wonderful LLC, Los Angeles, CA), [F]Bolthouse Bom Dia Acai-Mangosteen (Bolthouse Juice Products, LLC, Bakersfield, CA), [G]Trader Joe's Just Blueberry (Trader Joe's, Monrovia, CA), [H]Ocean-Spray-Pure Cranberry (Ocean Spray Cranberries Inc., Lakeville-Middleboro, MA), [I]TEAC (µM TE/mL).

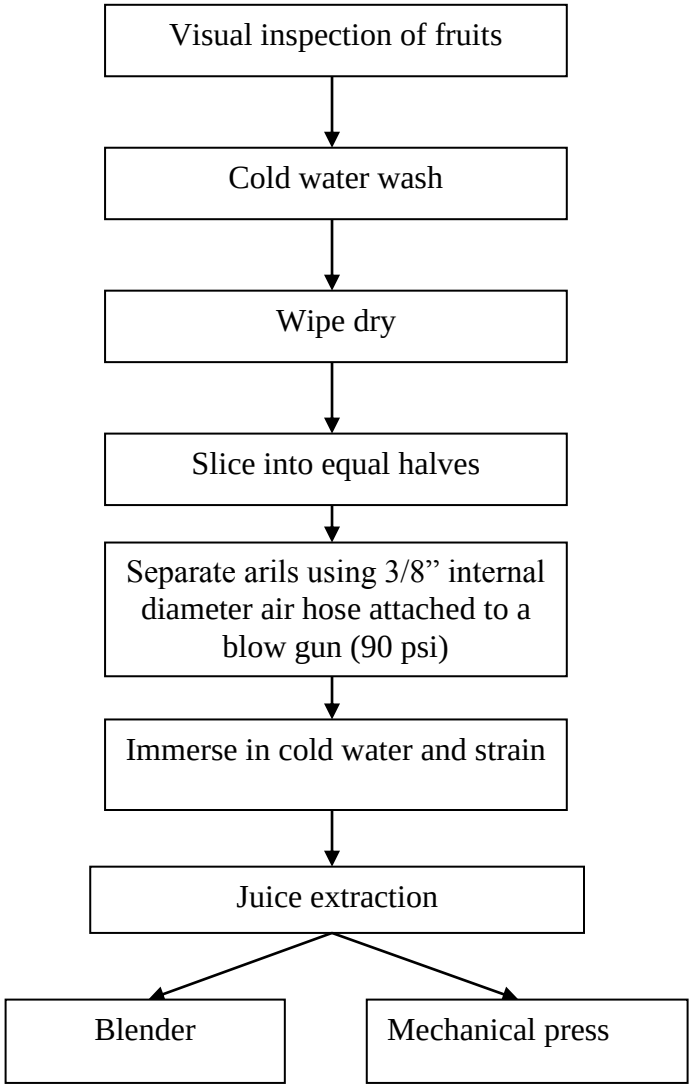
Visual inspection of fruits
Cold water wash
Wipe dry
Slice into equal halves
Separate arils using 3/8” internal diameter air hose attached to a blow gun (90 psi)
Immerse in cold water and strain
Juice extraction
Blender
Mechanical press

Figure 5.1a

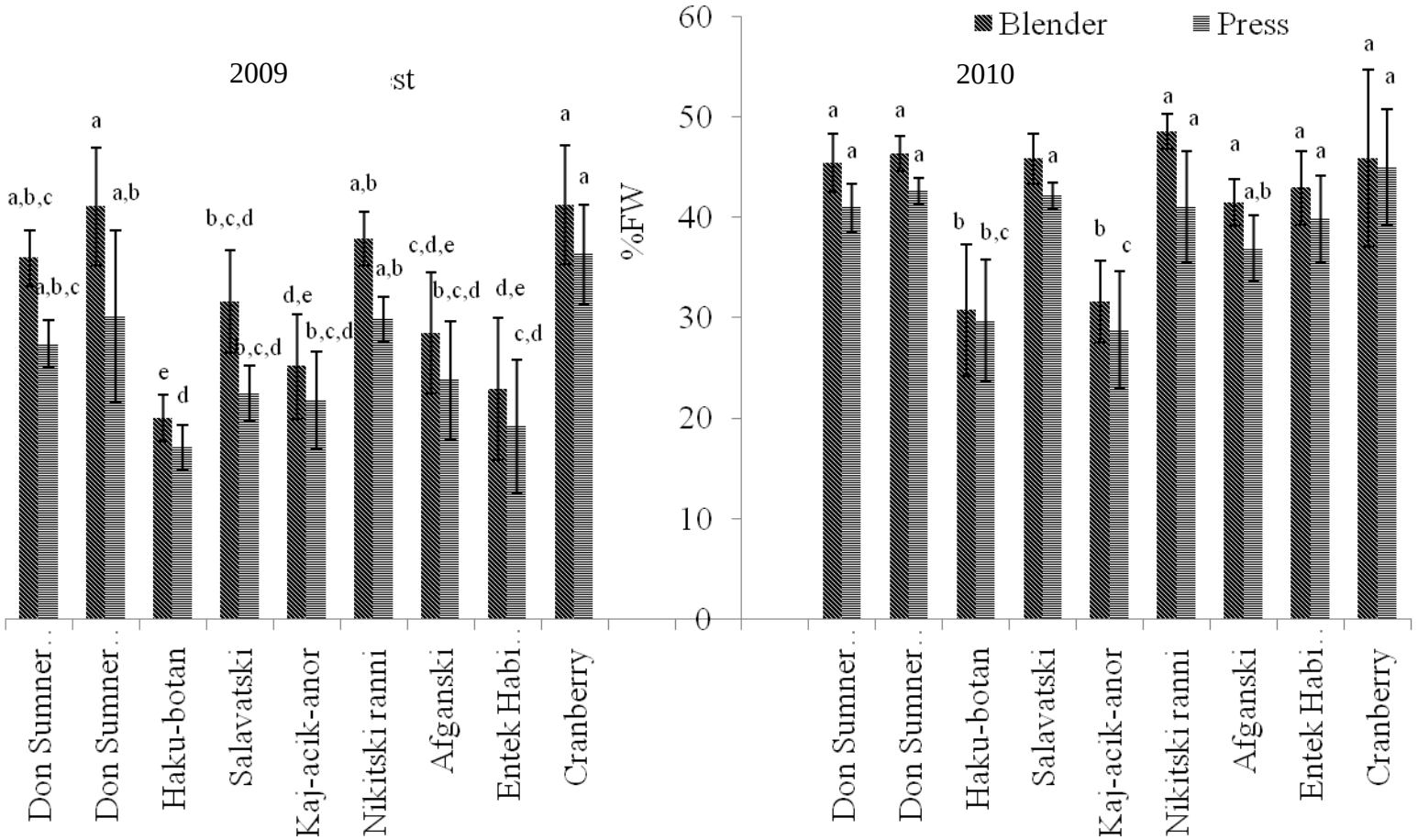
2009
2010
Blender
Press
%FW
60
50
40
30
20
10
0
Don Sumner...
Don Sumner...
Haku-botan
Salavatski
Kaj-acik-anor
Nikitski ranni
Afganski
Entek Habi...
Cranberry

Figure 5.1b

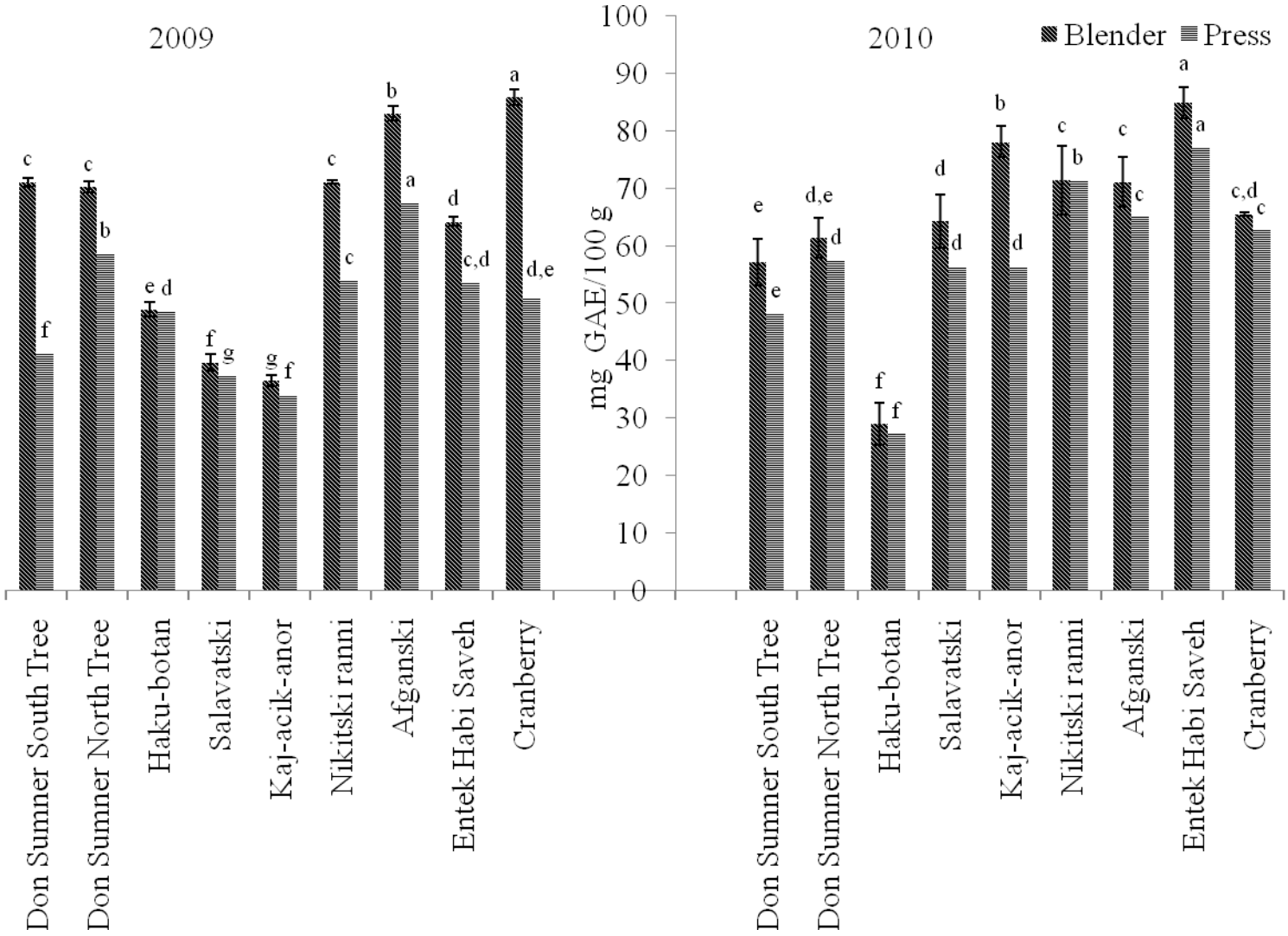
2009
2010
Blender
Press
mg GAE/100 g
100
90
80
70
60
50
40
30
20
10
0
Don Sumner South Tree
Don Sumner North Tree
Haku-botan
Salavatski
Kaj-acik-anor
Nikitski ranni
Afganski
Entek Habi Saveh
Cranberry

Figure 5.2

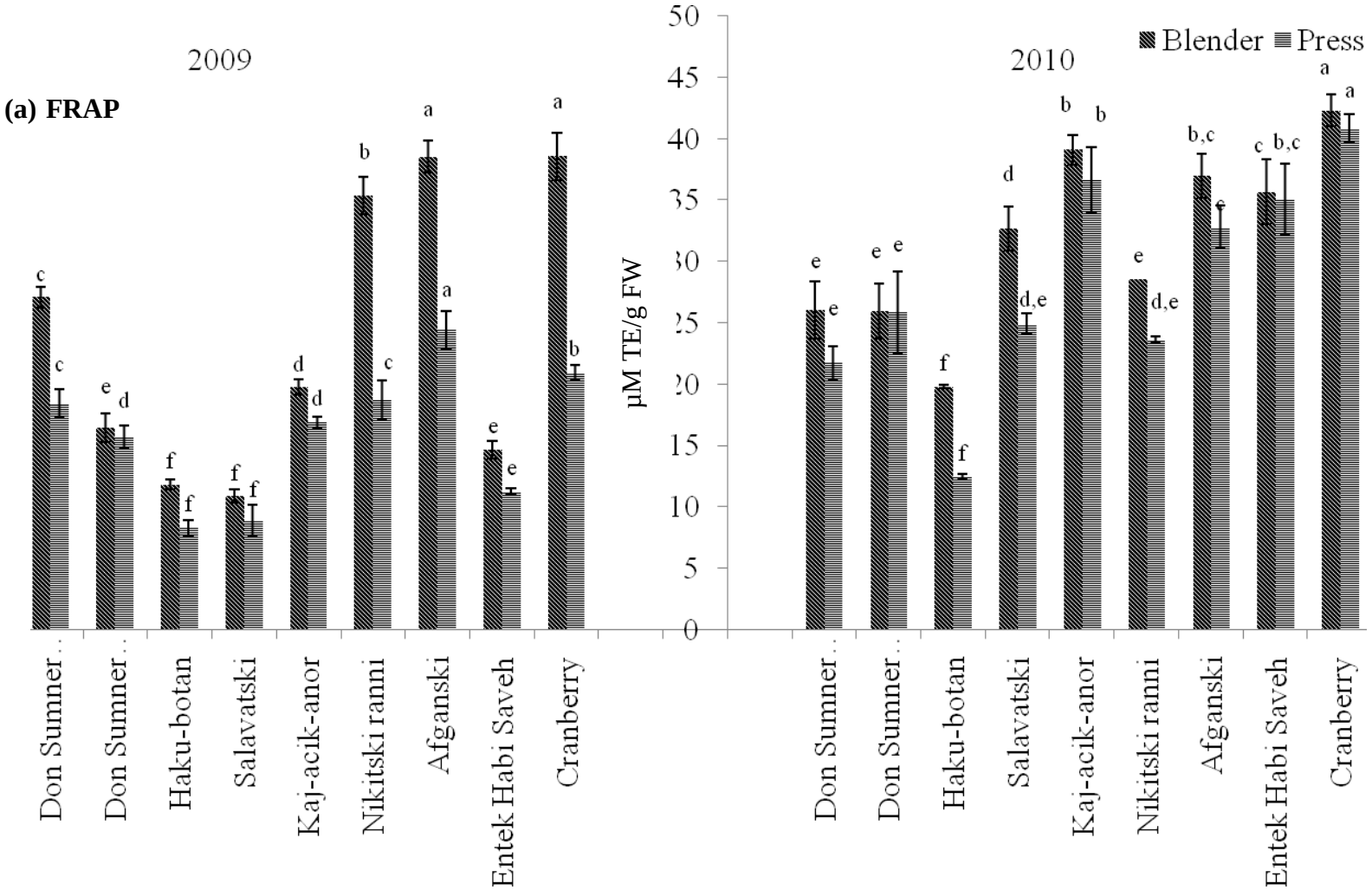
(a) FRAP
2009
2010
Blender
Press
µM TE/g FW
50
45
40
35
30
25
20
15
10
5
0
Don Sumner...
Don Sumner...
Haku-botan
Salavatski
Kaj-acik-anor
Nikitski ranni
Afganski
Entek Habi Saveh
Cranberry

Figure 5.3a

(b) TEAC

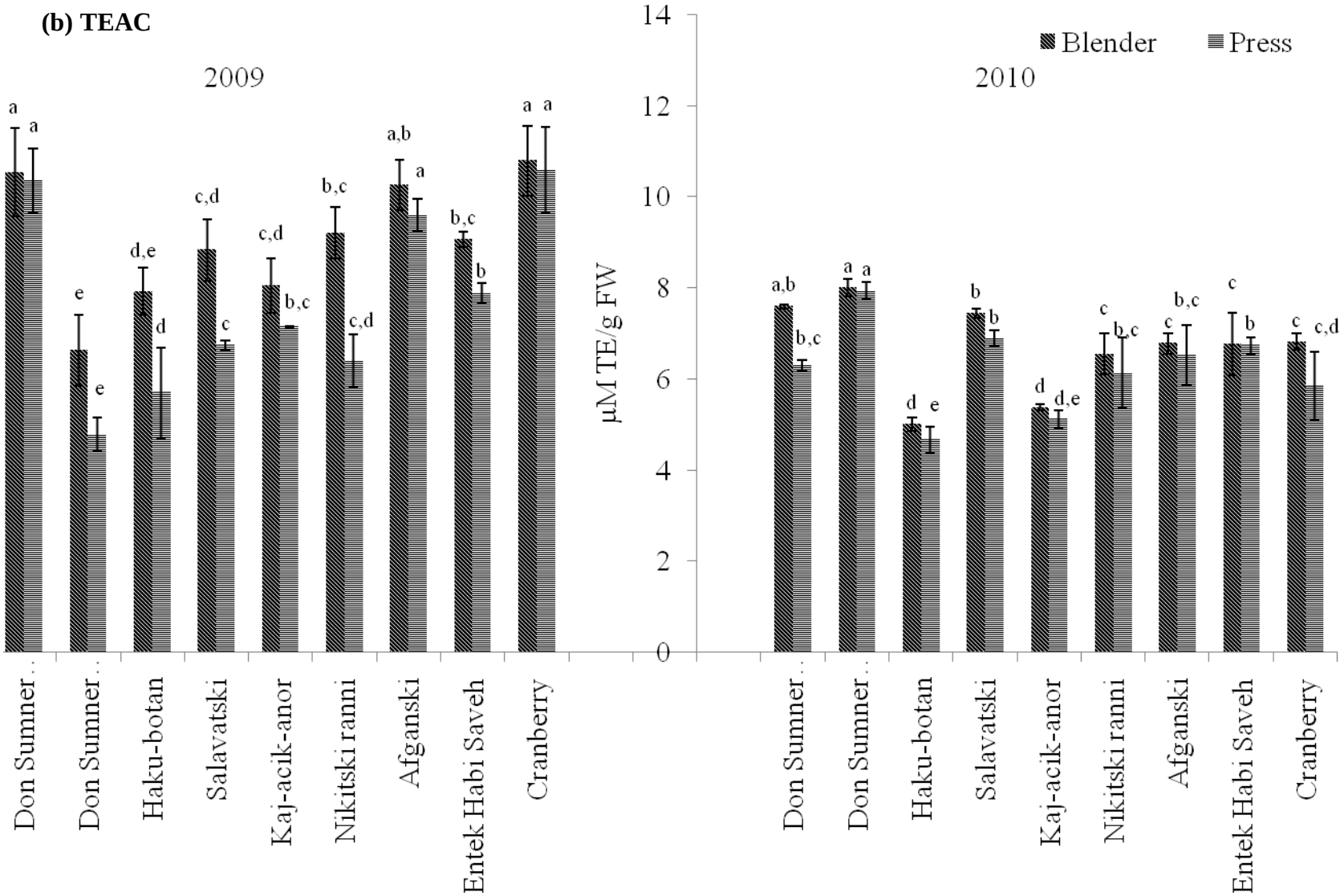

Figure 5.3b

(c) ORAC

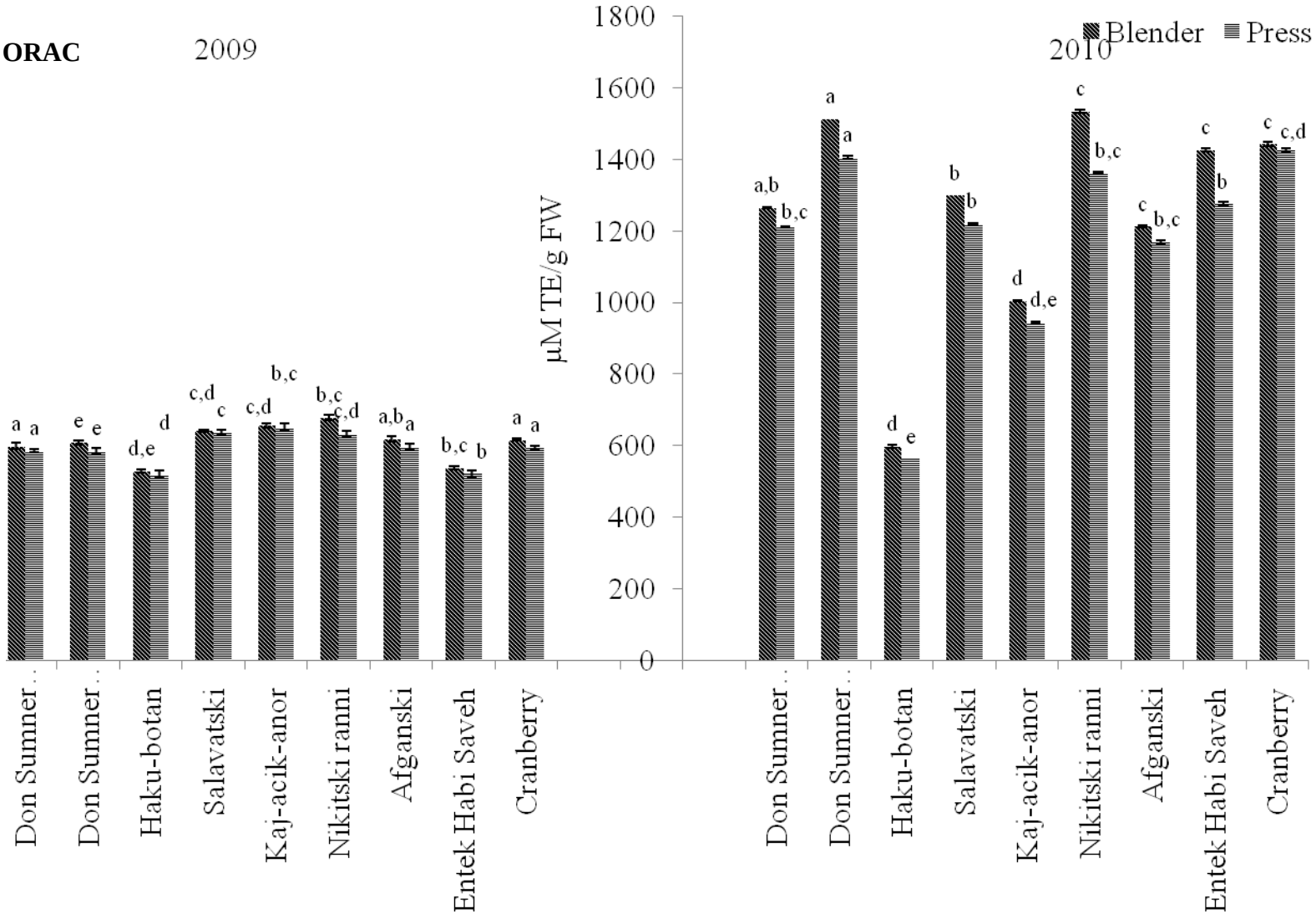

Figure 5.3c

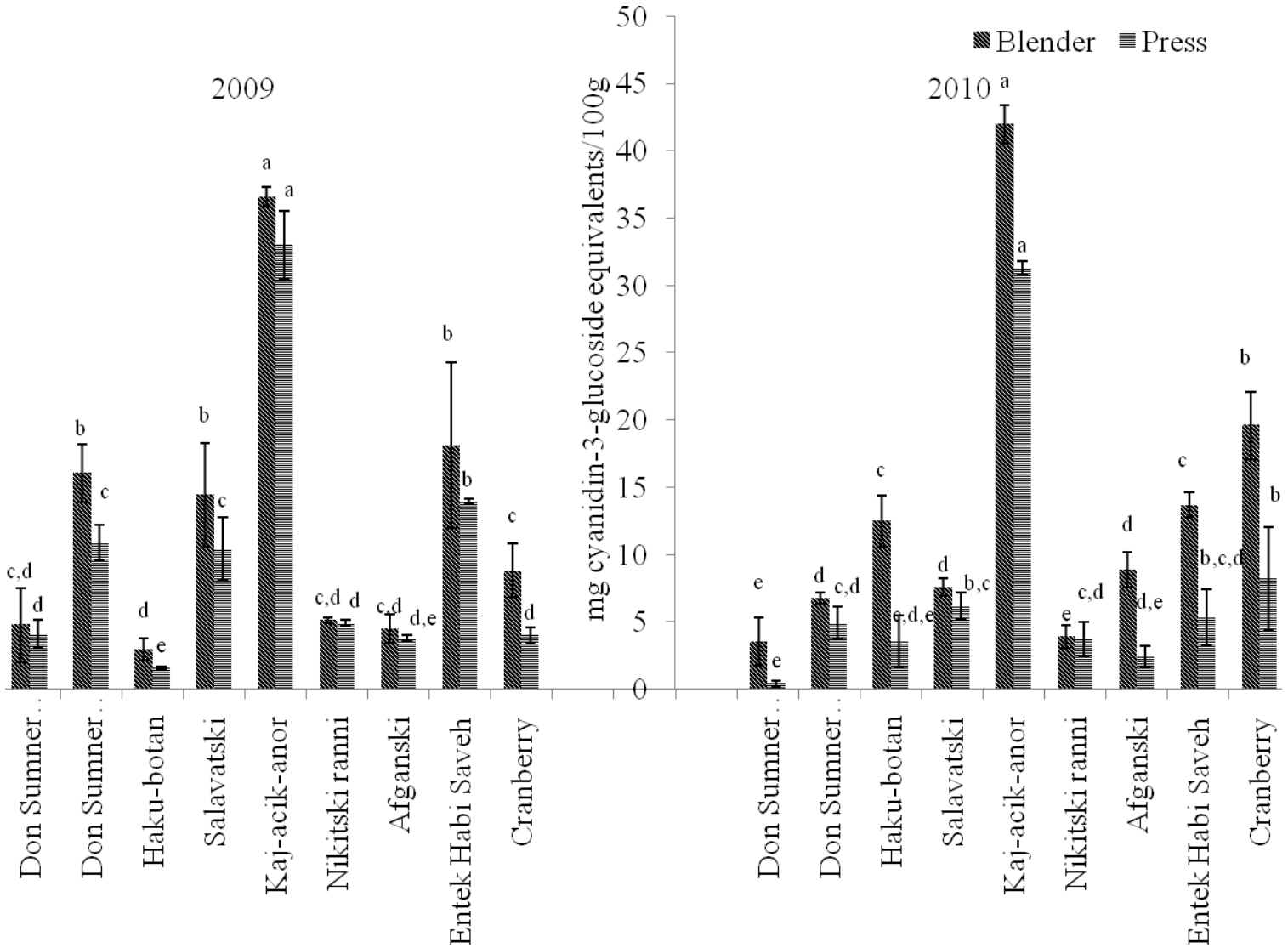
2009
2010
Blender
Press
mg cyanidin-3-glucoside equivalents/100g
0
5
10
15
20
25
30
35
40
45
50
Don Sumner...
Don Sumner...
Haku-botan
Salavatski
Kaj-acik-anor
Nikitski ranni
Afganski
Entek Habi Saveh
Cranberry
Don Sumner...
Don Sumner...
Haku-botan
Salavatski
Kaj-acik-anor
Nikitski ranni
Afganski
Entek Habi Saveh
Cranberry

Figure 5.4

CHAPTER 6

CONCLUSIONS

The pomegranate cultivars grown in Georgia varied in their phytochemical content. Significant differences among cultivars were observed for the chemical assays performed. Extracting the aril juice using two methods helped us identify and quantify changes in phenolic compounds, organic acids, and sugars. The yield was always higher for blender extracted juice and in the range of 20.01 - 51.16% FW. The total phenolic content was in the range of 28.88 - 84.94 mg GAE/100 g FW for cultivars obtained using both methods. Cultivar Cranberry had good antioxidant capacity compared to other cultivars. Cultivar Kaj-acik-anor had the highest anthocyanin levels. The major organic acid was citric acid, followed by malic acid. The major sugars detected in pomegranate juice were glucose and fructose. The individual phenolic compounds and organic acids profile can help in understanding the characteristic flavor and quality of juice.

The anthocyanins play an important role in the marketing of juice, since consumers associate intense red color to high quality product. The anthocyanin profile of pomegranate juice is unique. The major anthocyanin found was delphinidin 3-glucoside. The stability order of anthocyanins are malvidin >peonidin> pelargonidin>petunidin>cyanidin>delphinidin. Encapsulation techniques like spray drying could help stabilize anthocyanins. The maturity index, pH values, and total soluble sugars could be useful in characterizing the taste and flavor of the extracted juice. They can also serve as important parameters to select a highly nutritional fruit.

Significant correlations ($p \leq 0.05$) were found between the total polyphenols and antioxidant capacity, mainly FRAP method. However, classifying the juice based on visual color of the juice (light and dark) helped in better understanding the correlations, since differences were observed. The antioxidant methods were correlated to each other based on the visual color of juice. Therefore, it is important to report antioxidant capacity using at least two or more methods. The total monomeric anthocyanins were not correlated to the antioxidant capacity, indicating that they do not contribute as much to the juice's antioxidant capacity.

Blender extracted juice consistently had higher yield, total polyphenols, and antioxidant capacity. This may be due to the presence of seeds, pith, and carpellary membrane which contribute to the overall antioxidant capacity of the juice. As a result, it is a better and efficient method for extracting aril juice. Commercially, pomegranate juice is obtained by applying hydrostatic pressure to the whole fruit. A comparison of the total phenolic content and antioxidant capacity by TEAC for commercial pomegranate juice and other Georgia-grown crops was made in Table 5.4.

Comparison of yield, antioxidant capacity, total anthocyanins, and polyphenols between two different years, 2009 and 2010, suggested that climatic conditions play an important role in determining the nutraceutical profile of a pomegranate fruit. Based on the results, it is suggested that cultivars Cranberry and Kaj-acik-anor can be used for commercial production of pomegranate juice with high quality.

Future work

- Changes in phytochemical profile during maturation of fruits
- Study of the antioxidant/prooxidant activity in complex biological matrix and determine their effect on cancer cells
- Separation of individual phenolic compounds by different chromatographic techniques
- Sensory analysis of the pomegranate juice
- Extraction and purification of potential phenolic compounds like punicalagin and ellagic acid, and their effects on atherosclerosis.