BIOCHEMICAL ANALYSIS WITH MEASUREMENTS OF BIOACTIVE INGREDIENTS OF LEMON VARIETIES BIOHEMIJSKE ANALIZE IZMERENIH VREDNOSTI BIOAKTIVNIH SASTOJAKA SORTI LIMUNA

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ABSTRACT

The 'Sweet Lemon' and 'Interdonato' lemon varieties were evaluated based on their antioxidant activity, sugar, total phenol and citric, succinic and L-ascorbic acid content in the present study. The total phenol content (TPC) of 'Sweet Lemon' and 'Interdonato' lemon fruit juice were about 123.19 mgGAE/100g and 81.76 mgGAE/100g respectively. The FRAP content were detected as 146.86 μ mol Trolox/g in 'Sweet Lemon' while 106.78 μ mol Trolox/g in 'Interdonato' lemon variety. However the trolox equivalent antioxidant capacity (TEAC) assay, which measures the reduction of the ABTS radical cation by antioxidant ABTS content was 96.25 μ mol Trolox/g and 79.09 μ mol Trolox/g respectively. Fructose was 3.26 %, sucrose was 0.19 % and glucose was 2.44 % in 'Sweet Lemon' variety. Therefore, fructose content was 1.22 %, sucrose was 0.19 % and glucose was 0.80 % in 'Interdonato' lemon variety. The results indicated that 'Sweet Lemon' fruit juices are rich in FRAP, ABTS, TPC and fructose, succinic and L-ascorbic acid content compared to 'Interdonato' lemon. The organic acids (citric acid, L-Ascorbic acid and succinic acid) values of both 'Sweet Lemon' and 'Interdonato' are 0.24-8.86 %, 62.25-55.50 mg/100g and 1.93-0.09 %, respectively. The present study revealed that the healthy properties of lemon fruits have been attributed to L-ascorbic acid and phenolic compounds, mainly to antioxidant content.

Keywords: 'Sweet Lemon', 'Interdonato', sugar, phenol, antioxidant, L-ascorbic acids, organic acid.

REZIME

Sorte limuna „sweet lemon“ i „interdonato“ su ispitivane u studiji sa aspekta antioksidativne aktivnosti, sadržaja šećera, ukupnih fenola, kao i sadržaja limunske, sukcinске (čilibarne, butandionske) i L-askorbinske kiseline. Sadržaj ukupnih fenola (UF) soka sorti limuna „sweet lemon“ i „interdonato“ su iznosile oko 123,19 mg ekvivalenta galne kiseline /100g i 81,76 mg ekvivalenta galne kiseline/100g, redom. Metodom redukcije jona gvožđa (engl. FRAP) utvrđen je za sortu „sweet lemon“ sadržaj 146,86 μ mol Trolox ekvivalenta/g dok je za sortu „interdonato“ određeno 106,78 μ mol Trolox ekvivalenta/g. Test antioksidativnog kapaciteta Trolox ekvivalenta (engl. TEAC), kojim se određuje redukcija ABTS radikalskog katjona u prisustvu antioksidanasa, iznosila je 96,25 μ mol Trolox ekvivalenta/g i 79,09 μ mol Trolox ekvivalenta/g, respektivno. Sadržaj fruktoze je iznosio 3,26%, saharoze 0,19% i glukoze 2,44% u sorti „sweet lemon“. Za sortu limuna „interdonato“ sadržaj fruktoze je bio 1,22%, saharoze 0,19% i glukoze 0,80%. Rezultati ukazuju da je sorta „sweet lemon“ bogatija u sadržaju antioksidanasa određenih FRAP, ABTS i TPC metodama, kao i u sadržaju fruktoze, sukcinске (čilibarne, butandionske) i L-askorbinske kiseline u poređenju sa sortom limuna „interdonato“. Vrednosti sadržaja organskih kiselina (limunske, L-askorbinske i sukcinске (čilibarne, butandionske) kiseline) u sortama „sweet lemon“ i „interdonato“ su 0,24-8,86%, 62,25-55,50mg/100g and 1,93-0,09 %, redom. Ova studija je pokazala da pozitivni zdravstveni efekti sorti limuna mogu da budu vezani za L-askorbinsku kiselinu i fenolne komponente, uglavnom zbog antioksidantnog efekta.

Ključne reči: 'sweet lemon', 'interdonato', šećer, fenoli, antioksidanti, L-askorbinske kiseline, organske kiseline.

INTRODUCTION

Lemon fruit is important crop in the world which is cultivated in many region of the world especially at Mediterranean Countries. Turkey is one of the important producer with 725.230 t production among the Mediterranean Countries (Tuik, 2015). The size, the color and acidity of the fruit are important parameters to determine harvest time and the market price (Simon-Graoa et al., 2016). Fresh lemon fruit is commercially important for fresh products market and food industry which processed are as juices, jam, jellies, molasses, etc. Lemon fruit is an important as medicinal plant which is family of Rutaceae and important for food industry and health benefits because of their bioactive composition (González-Molina et al., 2010). Therefore the food industry uses phytochemical rich and high nutritive selected lemon cultivars (González-Molina et al., 2008). Lemon fruits are rich in alkaloids that have anticancer activities and the antibacterial potential in lemon leaves, stem, root and flower of (Kawaii et al., 2000). For this reasons chemical, cosmetic, pharmaceutical and food industry are used extracts of lemon bioactive

compounds like flavonoids, vitamins, minerals, dietary fiber, essential oils, etc. (Ozaki et al., 2000). Moreover organic acids, sugars and phenolic compounds are regarded main quality factors by both consumers and the food industry. Although the main important subject for selected of new citrus varieties used in the pharmacological and food technology area are their quality characteristics (Del Rio et al., 1997a; Horowitz and Gentili, 1977; Ortuno et al., 1997). Biochemical structures of lemons have bioavailability which useful in the chemoprevention or therapy of chemically induced human solid tumor cells across its activity as enzyme inhibitor, being a cancer chemo preventive such as skin cancer and colon cancer etc. (Reddy et al., 2003). Biochemical and bioactive ingredients fresh products are vary according to varieties, processing and irrigation methods etc. (Albertini et al., 2006). To our knowledge, there has been no previous publications on comparison 'Sweet Lemon' and 'Interdonato' lemon varieties based on their biochemical characteristics. For this reasons in the present study it was aimed to compare biochemical components and antioxidant activities of 'Sweet Lemon' and 'Interdonato' lemon varieties.

MATERIALS AND METHODS

Plant material

Lemon fruits were harvested commercially optimal stage in the experimental area of the private company orchard. After harvesting fruits were transferred to the laboratory were prepared for total antioxidant, phenolic, organic acids and sugar analysis. The methods used for the determine concentration of lemon fruit biochemical compounds often have a profound influence on the results.

Measurements of bioactive ingredients

The organic acid were measured by using an HPLC system (Agilent 1100 series G1322A; Germany) according to the method of Bozan et al., 1997. An Aminex HPX-87 H column (300 mm × 7.8 mm) was used. The organic acid was determined at a wavelength of 210 nm. Total phenol content (TPC) of the fruit juices was measured according to modified method of Spanos and Wrolstad (1990) by using Folin–Ciocalteu reagent. The samples absorbance were measured at 760 nm with the use of a Multiskan GO microplate spectrophotometer. The results were expressed as mg Gallic acid equivalent/g weight (mg GAE/g) (Bayır, 2007).

For the Trolox equivalent antioxidant capacity (TEAC) assay measures the reduction of the ABTS radical cation by antioxidant ABTS assay and the method of Re et al. 1999, were used with some small modifications. The results were expressed in μmol trolox equivalent (TE)/g fresh mass.

Method of Benzie and Strain, 1996, were used to for The ferric reducing antioxidant power (FRAP) assay was done according to the with some modifications. The results were expressed in μmol TE/g fresh mass.

The content of individual sugar content (glucose, fructose, sucrose) in fruit juice samples was measured using HPLC (Shimadzu) RID (Refractive Index) detector and aminex (300X7.8mm, 5m) column (Miron and Schaffer, 1991). The sugar contents of the samples were determined using the the refractive index detector.

RESULTS AND DISCUSSION

FRAP and ABTS assays were used for determined of the antioxidant activity in the two different lemon varieties. The results for the antioxidant activity of 'Sweet Lemon' and 'Interdonato' varieties were presented in Table 1. As seen in Table 1, the FRAP assay highlighted in both varieties than ABTS assay but 'Sweet' lemon has higher antioxidant activity than 'Interdonato' lemon variety. Similar results was found for anthocyanin content as well as for total phenolic acid content also. Comparing the both cultivars, 'Sweet' lemon variety had higher antioxidant activity with (146.86 μmol trolox/g), ABST was 96.25 μmol trolox/g and anthosianin content was 1.985 and total phenolic (g gallic acid/100g) acid content was 123,187 g gallic acid/100gext however 'Interdonato' lemon variety FRAP was 106,78 μmol trolox/g, ABST was 79.09 μmol trolox/g 0.86 mg/L and 81.76 g gallic acid/100g respectively (Table 1).

Corelation of the antioxidant activity and phenolic compound content varies on many parameters, both the chemical structure and use of chemical analysis methods etc.(Amenta et al., 2015). Phenolics and antioxidants are

important phytonutrients because they have biological effects that provide health-related properties. The organic acid composition of fruits is also interest because of its importantly influence on the sensory properties of fruits and fruit juices. One of the most popular natural source of ascorbic is lemon fruit which is benefit for human health moreover vitamin C has important effects on reduce of cardiovascular diseases, arteriosclerosis and risk of many chronic diseases (Amenta et al., 2015).

'Sweet Lemon' L-ascorbic acid content was 62.25 mg/100g but 'Interdonato' lemon had 55.5 mg/100g L-ascorbic acid as shown in Table 2. Another the major organic acids is citric acid in citrus juices. However 'Interdonato' lemon had highest citric acid with 8.86 % compared to 'Sweet' lemon with 0.24 %. Succinic acid content of 'Sweet Lemon' was 1.935 % and 'Interdonato' lemon was 0.09 %. Even though quantity of components in fruits are lower, combination of minor component with sugars, which are important for the sensory quality of raw and processed fruits (Wang et al., 1993).

The ratio of fructose, glucose and sucrose which are important carbohydrates are found in citrus fruit is usually 1:1:2 in. However in present study results showed little difference with literature as shown in Table 3.

It may results of variety and growing area also important effects on quality incidens. 'Sweet' lemon variety had highest sugar content compared to 'Interdonato' lemon (Tablo 3). As shown in Table 3, 'Sweet' lemon fructose contentent was 3.26 %, glucose was 2.44 % and sucrose was 0.19 % while 'Interdonato' lemon fructose was 1.22 %, glucose 0.80 % and sucrose was 0.185 %.

CONCLUSION

Lemon fruits are not only importance for health benefits but also their aroma, taste and nutritional values, combination of these components make fruits encouraged throughout world for health considerations. Therefore many studies evaluated lemon fruit is rich in biochemical and bioactive compounds as well as vitamins, minerals, dietary fiber, essential oils and carotenoids which are important for health-promoting.

Total phenolic content, vitamins (vitamin C) and antioxidants are rich in lemon fruits which are very important in human nutrition. The lemon fruits have a valuable levels of health beneficial phenol content, antioxidant activity, organic acids, sugars.

The present results showed that 'Sweet Lemon' variety was found rich in total phenols and anthocyanin contents, fructose,

Table 1. Antioxidant activity, anthocyanin content and total phenolic content of lemon varieties

Lemon Varieties	FRAP (μmol trolox/gext)	ABTS (μmol trolox/gext)	Anthocyanin content(mg/L)	Total phenolic (g gallic acid/100gext)
Sweet lemon	146.86 $\pm$ 8.1 ^A	96.25 $\pm$ 1.08 ^A	1.98 $\pm$ 0.35 ^A	123.187 $\pm$ 1.26A
Interdonato lemon	106.78 $\pm$ 7.9 ^B	79.09 $\pm$ 4.78 ^B	0.86 $\pm$ 0.34 ^B	81.76 $\pm$ 2.30B

Note: Data are the mean $\pm$ SE. Different letters within a rows show significant differences at $p < 0.05$.

Table 2. Lemon organic acids content

Lemon Varieties	Citric acid (%)	L-Ascorbic acid (mg/100mL)	Succinic acid (%)
Sweet lemon	0.24 $\pm$ 0.20 ^B	62.25 $\pm$ 4.5 ^A	1.93 $\pm$ 0.90 ^A
Interdonato lemon	8.86 $\pm$ 1.06 ^A	55.50 $\pm$ 4.0 ^A	0.09 $\pm$ 0.01 ^B

Note: Data are the mean $\pm$ SE. Different letters within a rows show significant differences at $p < 0.05$.

glucose, sucrose, succinic and L-ascorbic acid compare to the “Interdonato” lemon variety. Hereby results of the present study can be a luminary for new ideas for consumers, producers and plant growing, human health and nutrition working on this subject.

Table 3. Lemon sugar content

Lemon Varieties	Sucrose (%)	Glucose (%)	Fructose (%)
Sweet Lemon	0.19±0.1 ^A	2.44±0.23 ^A	3.26±0.1 ^A
Interdonato Lemon	0.185±0.05 ^A	0.80±0.10 ^A	1.22±0.2 ^B

Note: Data are the mean ± SE. Different letters within a rows show significant differences at $p < 0.05$.

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Received: 27. 02. 2017.

Accepted: 07. 06. 2017.