Quality of Pomegranate Juice from "Hicaz" Variety from North Macedonia with and without addition of sugar

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Abstract

The impact of the sugar on the chemical composition of pomegranate juice in terms of organic acids, total phenolics, catechins and anthocynanins from Macedonian "Hicaz" variety was objective of this study. The levels of all bioactive compounds were significantly higher to "Hicaz" pomegranate variety with addition of sugar and they were in good correlation to the results of hue and color of the juice. Although pigment stability and color changes depended on anthocyanin structure and juice matrix, sugars had significantly protective effects on the color disruption of freshly squeezed juice from pomegranates from the region of North Macedonia.

Key words: pomegranate juice from "Hicaz" variety, impact of sugar, anthocyanins, catechnines, total phenolic compounds

Introduction

Protection of foods from microbial spoilage using sugar (usually sucrose) has ancient roots and is often referred to as sugar curing**.** The most usual way of conservation of juices with sugars is simple osmosis, or dehydration. Sugar, whether in solid or aqueous form, attempts to reach equilibrium with the sugar content of the food product with which it is in contact. The sugar is added in juices to effectively increase both aroma and color retention and reduce the loss of TPC, anthocyanins, and other polypenolic compounds (Kopjar et al., 2008; Galmarini et al., 2009; Kopjar et al., 2009; Loncaric et al., 2014).

Regarding valuable bioactive compounds, rutin was predominant flavonoid from the pomegranates peel (Mphahlele *et al*., 2017). In the work of Atukuri et al., the pomegranates treated with fludioxonyl at 600mg/L had the best quality regarding decay incidence, weight loss, total phenolics and sensory attributes (Dak *et al*., 2014). The procedure of isolation and NMR elucidation of two new ellagitannin oligomers, pomegraniins A (tetramer) and B (pentamer), and a new glucose ester of neolignan, pomegralignan, together with six known ellagitannins from the arils and pericarps of *Punica granatum* L. (pomegranate) was studied by working group of Ito (Ito *et al*., 2014). Urolithins were separated from the intestinal metabolites of pomegranate ellagitannins by high-speed counter current chromatography. The interest of these compounds increased in the last years due to their possibility to reduce the oxidative stress status in colon cancer by decreasing the intracellular ROS and malondialdehyde levels, and increasing SOD activity in H_2O_2 treated Caco-2 cells (Zhao *et al*., 2015)

To the best of our knowledge, there is no published results for the impact of the sugar on the quality of pomegranates juice from "Hicaz" variety from the region of North Macedonia. Therefore, the objects of our study was impact of the sugar on the overall quality of freshly squeezed juice from "Hicaz" variety or more precisely, the effect of sugar on bioactive compounds such anthocyanins, catechins and other polyphenolics.

Materials and methods

Sample preparation

The sampled fruits were selected randomly in order to separate three replicates for analysis, using 10 kg per replicate and cultivar. Pomegranates from "Hicaz" variety were weighed, cut in halves, and arils were hand separated from the pith avoiding contamination by components in membranous walls (septum). Juices of each cultivar were obtained by pressure of arils and were weighed to determine the juice yield. Samples of freshly prepared juice were stored frozen (−20ºC) until analyzed (O' Grady *et al*., 2014).

Determination of titratable acidity, total soluble solids, pH

For determination of titratable acidity (TA), 2 ml of fresh juice was diluted with 70 mL of distilled water and titrating with 0.1 M NaOH to an end point of pH 8.2 using a Metrohm 862 compact titrosampler (Herisua, Switzerland). The results were expressed as percentage of citric acid (% CA). Total soluble solids (TSS, °Brix) was measured using a digital refractometer (Atago, Tokyo, Japan) calibrated with distilled water. The pH values were determined at room temperature using a calibrated pH meter (Crison, Model 00924, Barcelona, Spain). All measurements were made on triplicate.

Determination of organic acids

For determination of organic acids, A Chromatograph Agilent technologies 1200 series, with Jasco AS-950 sampler, an auto injector (10 µl injection volume) and refractive index detector was used for analyses of organic acids. Separation of organic acid was performed on AMINEX HOX-87 (H 300 x 7.8) column. The eluent was prepared by mixing of 75µl H_2SO_4 in 250 mL H_2O. The mobile phase flow rate was 0.6 mL/min and the temperature was 55°C.

The percentages of organic acids were measured by following calculation:

$$mg\ organic\ ac./100ml = \frac{At}{As}\ x\frac{Cs}{Ct}\ x100$$

where:

- **At** is the area of test sample
- **As** is the area of standard
- **Cs** is the concentration of standard in mg/mL and
- **Ct** is the concentration of test in mg/mL

Determination of total phenolic compounds (TPC), total catechins and total antocynanins

The total phenolic content of pomegranate juices was determined with Folin–Ciocalteau reagent (O' GRADY et al., 2014). Determination of total phenolics was performed by the colorimetric method of Singleton and Rossi (SINGLETON and ROSSI, 1965). For determination of total catechins, the modified method of Atamossa and Gholap was used. In brief, the pomegranate juices from both varieties were dissolved in water (1:5) and measurements of total catechins was performed using UV-VIS spectrometer in the spectral range of 200 to 500 nm (ATAMOSSA and GHOLAP, 2015). The measurements were performed in triplicate.

Statistical analyses

The level of significance in differences between anthocyanin content and total phenolic content was determined by 5% by a one-way ANOVA using Tukey's test. The results from statistical analyses were classified using letters (different letters means significant differences among results). The letters are a and b according to the decrease of the result values. SPSS v.16.0 software, IBM corporation, USA was used for the applied statistical treatment.

Results and discussions

The chemical parameters of the samples of pomegranate juices from "Hicaz" variety with and without addition of sugar are presented in table 1.

Table 5. Chemical parameters of pomegranate juice

Samples of fresh juice from "Hicaz" variety	Brix	pH	Total acids g/L	Malic acid g/L	Citric acid g/L	Total phenolics	Total catechines	Total antocynains
Fresh juice without sugar addition	15.8±0.2[b]	3.07±0.1[a]	27.5±1.1[a]	0.18±0.03[a]	0.28±0.02[b]	2457±228[b]	25.9±2.1[b]	369.9±27.1[b]
Fresh juice with sugar addition	18.7±0.6[a]	3.07±0.0[a]	23.4±0.4[b]	0.17±0.05[a]	0.40±0.06[a]	3367±321[a]	44.9±0.8[a]	598.3±31.9[a]
	IC	**Hue**	**A_{420}**	**A_{520}**	**A_{620}**			
Fresh juice without sugar addition	2.81±0.11[b]	0.22±0.07[a]	17.1±0.08[b]	72.4±3.18[a]	9.8±0.3[a]			
Fresh juice with sugar addition	5.15±0.41[a]	0.33±0.05[b]	22.5±0.13[a]	67.9±5.11[b]	9.5±0.2[a]			

As we can see, the addition of sugar strongly affected all measured parameters with exception of pH and malic acid of the juice. The level of citric acid was significantly lower in juice without addition of sugar. The same tendency is continued for all bioactive compounds with highest effect on catechnins and anthocyanins Furthermore, the amount of monomeric anthocynains from "Hicaz" variety without sugar addition was very similar to the results published for pomegranate juices from the working group of Jaiswal (Jaiswal *et al*., 2010). The intensity of the color was almost double for juice with sugar, which was expected, due to the higher level of total phenolic compounds and monomeric anthocyanins (Table 1). Moreover, the higher intensity of red color can be linked to the dominance of cyanidin-3-glucoside as the major anthocyanin in pomegranate juice Cassano *et al*.,

2011; Varasteh *et al*., 2012). Furthermore, in the excellent article of Song et al., the effects of sugars on the color stability of fresh blueberry juice were investigated. According to their results, sugars decreased color in a concentration-dependent manner. However, glucose and galactose had significantly protective effects on the color disruption than fructose, maltose, and sucrose. Also, the author stated that organic acids lowered the color intensity in the order of citric acid> tartaric acid> malic acid> formic acid> acetic acid during 10 days of storage (Song et al., 2018). According to the statement of Sadilova et al., the impact of sugar on retention of anthocynains and their color properties depends of type of juice. Pigment stability and color changes depended on anthocyanin structure and juice matrix. Manually squeezed juices from fresh plant material showed higher color stability compared to juices prepared from concentrate which might be due to the retention of polymeric matrix compounds in the former (Sadilova et al., 2009). As we can see from the table 1, the highest impact of the sugar was detected from the yellow and red color. For matrix of pomegranate juice from "Hicaz" variety, the effect of the sugars on blue color was statistically insignificant.

The pomegranate juice from Macedonian "Hicaz" variety without sugar had higher value for total phenolic components than Valencia variety but significantly lower value than Akko, Hershkovitz and Wonderful varieties (Jaiswal *et al*., 2010). The amount of total phenolic component in pomegranate juice was similar to total phenolic content in Spanish pomegranate juice which was in range from 170-270 mg/mL gallic acid equivalent (Galindo *et al*., 2014).

Conclusion

The purpose of the present study was to evaluate the impact of the sugar on the quality of freshly squeezed juice from "Hicaz" pomegranate variety from the region of North Macedonia as well as its effect on bioactive compounds such catechins and anthocyanins. Based on the explanation above, we can summarize that pomegranate juice from "Hicaz" variety with addition of sugar is richer source with polyphenolic compounds in particular monomeric anthocyanins and total phenolic compounds. Our study showed the highest impact of the sugar on the yellow and red color. For matrix of pomegranate juice from "Hicaz" variety, the effect of the sugars on the blue colour was statistically insignificant. Results indicate that sugar addition before processing could have potential for enhancing product quality.

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