



**AgroSym**  
2019

**BOOK OF  
PROCEEDINGS**



*X International Scientific Agriculture Symposium  
"AGROSYM 2019"  
Jahorina, October 03-06, 2019*

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## FOREWORD

### *A Word from the Editor-in-Chief*

Dear colleagues,

In your hands is the Book of Proceedings of the X International Scientific Agricultural Symposium “AGROSYM 2019”, which I hope you will find useful in your work. As many as 900 contributions, from 82 countries, have been accepted for oral or poster presentations. Symposium themes cover all branches of agriculture and are divided into 7 sessions: 1) Plant production, 2) Plant protection and food safety, 3) Organic agriculture, 4) Environmental protection and natural resources management, 5) Animal husbandry, 6) Rural development and agro-economy, 7) Forestry and agroforestry. Papers dealing with agricultural engineering and technology were included into one of the seven sessions depending on their focus.

In the plenary lectures were addressed interesting topics; one keynote was on biotechnology and two others dealt with organic farming in Australia and Europe. This confirms the role of AGROSYM as a forum for open discussions and exchanges on agriculture, food, the environment and rural development in the Balkans and beyond. Many of the papers identify a number of approaches and market-based incentives to encourage producers to achieve higher levels of performance (from both economic and environmental points of view) and as a result to meet the expectations of governments and consumers.

The successful management of agricultural resources to satisfy changing human needs, while maintaining or enhancing the quality of the environment and conserving natural resources, indicate a long-term agricultural development imperative. Advances in productivity, profitability and stability of modern cropping, animal and forestry systems will have to be achieved globally on an ecologically sustainable basis. Today, it is obvious that conventional methods of agricultural production, while providing sufficient food and various products to humanity, have led to a number of negative impacts, including the transgression of many planetary boundaries. These negative impacts raise serious questions about the long-term sustainability of high-input agriculture and call for a genuine transition towards sustainable agro-food systems, which achieve food and nutrition security for present and future generations within the safe operating space for humanity.

Full texts of the submitted communications will be available on the website of AGROSYM (<http://agrosym.ues.rs.ba>). Each paper included in the present Book of Proceedings was positively reviewed.

Much appreciation is due to the authors of all papers submitted and presented at the symposium as well as to all symposium participants whose ideas and contributions allowed rich and lively discussions during the various sessions. Many thanks to all reviewers, session moderators and colleagues for their help in editing the Book of Proceedings. Special thanks go to all co-organizers, partners and sponsors for their unselfish collaboration and comprehensive support.

Editor-in-Chief



Dusan Kovacevic, PhD

East Sarajevo, 12 October 2019

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## **IMPACT OF PASTERIZATION ON THE QUALITY OF POMEGRANATE JUICE FROM "HICAZ" VARIETY FROM THE REGION OF NORTH MACEDONIA**

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### **Abstract**

Originating from Iran and the surrounding area, pomegranates have been cultivated for thousands of years yet are still considered emerging crops although well noted for ancient uses and cultural importance. Pomegranates contain a number of functional compounds that are responsible for beneficial health properties. Polyphenolic compounds are primarily responsible for these benefits and include phenolic acids, flavonoids (e.g. anthocyanins), and tannins. These compounds have shown effects in many studies including those related to cardiovascular, anti-inflammatory, anticancer, and antidiabetic conditions. The object of our study was to investigate the impact of pasteurization on the quality of pomegranate juice from "Hicaz" variety from the region of North Macedonia. The pH value and total acidity of the juice were not affected by pasteurization. There were no significant differences between those two parameters in fresh and pasteurized juice. However, all other parameters were significantly affected. The level of citric acid was almost double in fresh juice from "Hicaz" variety of pomegranate (781 mg/L) in comparison with pasteurized juice (402 mg/L). The amount of total anthocyanins was higher in fresh juice (598 mg/L) in comparison to pasteurized juice (537 mg/L). In addition, total phenols were higher in fresh then pasteurized juice (3367 mg/L and 3196 mg/L respectively). Opposite to this tendency, the total catechins were higher in pasteurized (50.1 mg/L) than fresh juice (44.9 mg/L). The pasteurization strongly affected the color of juice. The intensity of the color, the hue and the yellow color were higher in fresh juice. Opposite, the red and blue colors were more intense in pasteurized juice. The results from our study lead us to conclusion that pasteurization influenced significantly the quality of pomegranate juices from "Hicaz" variety.

**Key words:** *pasteurization, organic acid, total phenolic compounds, anthocyanins, catechins.*

### **Introduction**

Pomegranate (*Punica granatum* L.) is one of the major sources of polyphenolic phytochemicals, such as anthocyanins and catechins (Mphahlele *et al.*, 2014; Jaiswal *et al.*, 2010). However, the processing and storage of pomegranate juice had a decisive impact on the degradation of anthocyanin compounds and the consequent formation of brown pigments (Vegara *et al.*, 2013). Total anthocyanin content and antioxidant capacity were substantially and significantly influenced by the heat treatment applied according to the working group of Mena (Mena *et al.*, 2013). The results from study of Tezcan *et al.* showed that commercial pomegranate juices had markedly high total phenolic contents and antioxidant capacity. In the six commercial pomegranate juices studied in their work, in comparison to fruit juices reported in the literature, much higher total phenolic content and antioxidant capacities were observed, with increased health benefits for the consumers (Di Nunzio *et al.*, 2013). The antioxidant activity of pomegranate aril juice, attributed to a great extent to total phenols and anthocyanins by cyanidin-3,5-diglucoside was the major antocyanin in pomegranates. (Cassano *et al.*, 2011; Turfan *et al.*, 2011). Furthermore, excellent review of Kalaycioğlu and Erim quantitatively established the antioxidant activity, total phenolic content, anthocyanins,



organic acids, sugars and other important ingredients in pomegranate juices obtained from cultivars from different regions (Kalaycıoğlu and Erim, 2017). The late-pomegranate fruits were rich in phytochemicals and could be of great interest to the juice industry (Galindo *et al.*, 2014; Nag and Sit, 2018). In the work of Mena *et al.*, 15 pomegranate cultivars was studied in order to demonstrate the wide diversity among the quality of Spanish pomegranates. According to their findings, "Wonderful" juices displayed large antioxidant activity and a polyphenol content with very high acidity. In contrast, 'Mollar de Elche' showed fewer anthocyanins although it had very superior organoleptic properties. In addition to a high content in ellagitannins, 'Valenciana' juices had exclusive colour parameters (Mena *et al.*, 2011). Regarding total phenol content, rutin was predominant flavonoid from the pomegranates peel (Mphahlele *et al.*, 2017).

To the best of our knowledge, there is no published results for the impact of the pasteurization on the quality of pomegranate juices from the region of North Macedonia. Therefore, the objects of this study was impact of pasteurization on freshly squeezed juice from "Hicaz" pomegranate variety as well as determination of chemical parameters such total polyphenolics, total anthocyanins, the level of organic acids as well and intensity of color and hue.

### **Materials and Methods**

#### ***Sample preparation***

The sampled fruits were harvested from the region of Kavadarci and Valandovo (East region of North Macedonia in October 2018. The samples were selected randomly in order to separate three replicates for analysis, using 10 kg per replicate and cultivar. Pomegranates were weighed, cut in halves, and arils were handseparated 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 (−20 °C) until analyzed (O'Grady *et al.*, 2014). The process of pasteurization was performed on 65°C for 20 min.

#### ***Determination of titratable acidity, total soluble solids, pH***

All analyses for the quality of pomegranate juices as well as the process of pasteurization were performed at the department of food control at UNILAB, Faculty of Agriculture, University "Goce Delčev"-Štip.

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<sub>2</sub>SO<sub>4</sub> in 250 mL H<sub>2</sub>O. 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:

$$mg\ organic\ ac./100ml = \frac{A_t}{A_s} \times \frac{C_s}{C_t} \times 100$$

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

### 1.1.Determination of total phenolic compounds (TPC), colour, total catechins and total antocyanins

The total phenolic content of pomegranate juices was determined with Folin–Ciocalteu 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.

### 1.2.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,b,c,d,e and f 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 Discussion

The chemical parameters of the samples of fresh and pasteurized pomegranate juice from "Hicaz" variety are presented in table 1.

Table 1. Chemical parameters of pomegranate fresh and pasteurized juice

Samples of "Hicaz" juice	Brix	pH	Total acids g/L	Malic acid g/L	Citric acid g/L	Total phenolics	Total catechins	Total antocyanins
Fresh	16.0±1.1 <sup>a</sup>	3.04±0.0 <sup>a</sup>	29.9±2.3 <sup>a</sup>	0.18±0.10 <sup>b</sup>	0.28±0.03 <sup>b</sup>	2422±211 <sup>b</sup>	25.5±4.3 <sup>b</sup>	362.2±28.2 <sup>b</sup>
Pasteurized	16.8±0.8 <sup>a</sup>	3.04±0.1 <sup>a</sup>	24.3±1.1 <sup>b</sup>	0.36±0.52 <sup>a</sup>	0.78±0.09 <sup>a</sup>	3196±207 <sup>a</sup>	50.1±5.0 <sup>a</sup>	537.6±31.8 <sup>a</sup>
	IC	Hue	A420	A520	A620			
Fresh	2.87±0.12 <sup>b</sup>	0.23±0.07 <sup>a</sup>	15.7±0.12 <sup>b</sup>	72.9±5.98 <sup>a</sup>	9.9±0.5 <sup>a</sup>			
Pasteurized	4.14±0.27 <sup>a</sup>	0.26±0.05 <sup>a</sup>	19.6±0.22 <sup>a</sup>	70.5±4.77 <sup>a</sup>	9.8±1.4 <sup>a</sup>			

\*Source: Author's elaboration based on the obtained results.

As results show, there is significant difference between all measured parameters with exception of pH of the juices and brix. The level of malic and citric acid was significantly lower in comparison to commercial pomegranate juices from Turkey. However, the amounts of total phenolic compounds for fresh "Hicaz" juice was very similar to the same samples (Di Nunzio *et al.*, 2013). The level of monomeric anthocyanins were almost double for pasteurized in comparison to fresh juice. Furthermore, the amount of monomeric anthocyanins for fresh juice 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 higher for pasteurized juice, 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 (Turfan *et al.*, 2011; Cassano *et al.*, 2011; Varasteh *et al.*, 2012). Furthermore, citric and malic acids were the predominant organic acids in the sample of pasteurized juice. The acids attributed to the formation of ester upon the reaction of predominant citric acid with some juice compounds (Gundogdua and Yilmaz, 2012).

The results for amounts of total phenolic compounds obtained from Macedonian variety of "Hicaz" are similar to some varieties published by working group of Mena. (Mena *et al.*, 2011). According to their findings, total phenolic content in WSN sample had similar value as Macedonian "Hicaz" variety. He stated that Folin–Ciocalteu values were not the sum of polyphenols which can be detected by HPLC (EA derivatives, punicalagins and anthocyanins) as other compounds, such as flavanols, flavonols, phenolic acids, proanthocyanidins, and hydrolysable tannins that are different to punicalagins, also contribute to the phenolic profile (Mena *et al.*, 2011). The pomegranate juice from Macedonian "Hicaz" variety 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 for fresh juice is 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).

Generally speaking, we assumed that higher level of anthocyanins, catehines, total phenolic compounds and intensity of the color in pasteurized juice can be results of enzymes inactivation and microbial destruction (Reddy *et al.*, 2007). Results from our study showed that pH 3 is insufficient to inactivate enzymes and microbiota in degradation of phenolic compounds in fresh juice. However, changes of the color can be linked by the degradation of anthocyanin compounds and the consequent formation of brown pigments (Vegara *et al.*, 2013).

### **Conclusion**

The purpose of the present study was to evaluate the overall quality of freshly squeezed and pasteurized juice from "Hicaz" pomegranate variety from the region of North Macedonia. Based on the explanation above, it can summarize that pasteurized pomegranate juice from "Hicaz" variety is richer source with polyphenolic compounds in particular monomeric anthocyanins and total phenolic compounds. The effect of pasteurization gives improvement in terms of the enzymes inactivation and microbial destruction, which affects to the chemical parameters and quality of the juice.

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