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Antioxidant and Antimicrobial Capacity of Greek Commercial Pomegranate -Based Juices

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Abstract: *Pomegranates have health-promoting benefits because of their polyphenol constituents. The aim of this study was the description of some quality characteristics of five commercial pomegranate-based juices. Specifically the total phenolic content and the antioxidant capacity were determined using the Folin-Ciocalteu method and the DPPH method respectively as well as the antibacterial activity of the juices against pathogens e.g. E. coli, S. aureus and L. monocytogenes. The results showed that natural pomegranate juice contains less phenolics than blackberry and strawberry juice. On the contrary, grape juice contains less phenolic compounds than pomegranate juice from concentrate. It seems that juices from concentrate had a higher phenolic content than the natural juices. All juices showed high antioxidant capacity and the correlation between antioxidant activity and total phenolic content was confirmed. The majority of the juices induced a remarkable growth inhibition of the pathogens and namely those from concentrate exhibited the strongest antibacterial activity.*
Keywords: *Pomegranate-based juice, antioxidant capacity, antimicrobial capacity*

I. Introduction

Pomegranate (Punica granatum L.) belongs to the family Punicaceae which includes only one genus and two species, the other one, little-known, being P. protopunica Balf. peculiar to the island of Socotra in the Indian Ocean [1]. Pomegranate is one of the oldest edible fruits. Wild pomegranates are growing today in central Asia from Iran and Turkmenistan to northern India where they are considered as natives. Other researchers determined Near East, Southwestern Asia, or Iran and its surroundings as its origin [2]. Nowadays, pomegranate is an important commercial fruit crop that is widely cultivated in parts of Asia, North Africa, the Mediterranean, and the Middle East [3]. Pomegranates are also growing in the USA [2]. In Greece, pomegranate is known since ancient times and is related with mythology and tradition. Recently it has become a growing cultivation. The edible part of pomegranate fruit can be consumed fresh or used for the preparation of fresh juice, canned beverages, jelly, jam and paste and also for flavoring and coloring beverage products [4]. Pomegranate juice can be found as “juice from direct extract”, “juice from concentrate”, “juice concentrate” and “juice from concentrate with natural flavors” [5]. Before shipping to their final destination, fruit juices are concentrated to ensure longer storage life and easier transportation. The concentration involves partial removal of water leaving all the original solid components such as sugars, minerals and vitamins to the more concentrated solution. Therefore, concentration is of critical importance as it determines the quality of the final product such as flavor, color and appearance [6].

Because of the low aromatic intensity of pomegranate and the processing used to obtain the juice, sometimes the flavors of commercial products are not similar to the original fruit. This fact has a significant impact on the juices’ acceptance by consumers. One of the most popular ways of improving their quality or shelf life is the addition of sugars or juices made from other fruits with attractive colors and aromas [7].

There is a growing interest in this fruit because it is considered to be a functional product of great benefit in the human diet as it contains several groups of substances that are useful in disease risk reduction. Epidemiological studies show that consumption of pomegranate correlates with reduced cardiovascular and cerebrovascular diseases, anti-diabetic and anti-inflammatory activity and reduced cancer mortality [8-19]. Other studies suggest that some compounds of the pomegranate juice are characterized by antioxidant, antimicrobial and antiviral activities [20-25]. The beneficial health qualities have been attributed to the exceptionally high antioxidant capacity of the fruit juice, seemingly the result of the remarkably high content and unique composition of soluble phenolic compounds [26,27]. It has been reported that pomegranate juice is one of the important sources of anthocyanins (cyanidin, delphinidin, and pelargonidin), which give the fruit and aril its red colour, and some of the phenolics and tannins (such as punicalin, pedunculagin, punicalagin and ellagic acid) [28]. The principal antioxidant polyphenols in pomegranate juice include the ellagitannins and anthocyanins [25, 29].

The objectives of this study were (1) to determine antioxidant activities and total phenolic contents of commercial pomegranate-based juices and (2) to evaluate the antibacterial effect of these juices on the growth inhibition kinetics of *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*.

II. Materials And Methods

2.1 *Chemicals*

Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, gallic acid and methanol were purchased from Sigma Aldrich (Germany). Tryptic soy broth (TSB) was obtained from Biolife Italiana (Italy). Agar was purchased from Carl Roth (Germany).

2.2 Samples

Five commercial pomegranate-based juices [natural pomegranate juice (PJ), natural pomegranate-blackberry juice (PBJ), natural pomegranate-strawberry juice (PSJ), pomegranate juice from concentrate (PFCO) and pomegranate-grape juice from concentrate (PGFCO)] were used for the study. The PBJ, PSJ and PGFCO samples contained pomegranate juice mixed with blackberry, strawberry and grape juice respectively in ratio 50:50. All these products were produced in Greece and were purchased from local supermarkets. The PJ, PBJ and PSJ belonged to one commercial trademark and PFCO and PGFCO belonged to another. They were stored following the instructions on the packaging and studied before the indicated expiry date.

2.3 Determination of pH

Pomegranate based juices' pH was measured with a pH-meter (Jenway, model 3305).

2.4 Determination of Total Phenolics (TPs)

Total phenolics were determined by using the Folin-Ciocalteu method [30]. The PFCO and PGFCO juices were diluted in ratio 1:80 with methanol:water (6:4) while the rest of the juices were diluted in ratio 1:20. 300 μl of diluted juice was mixed with 1.5 ml of 10-fold-diluted Folin-Ciocalteu reagent and 1.2 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 90 minutes at room temperature before the absorbance was measured by an Anthelie UV-vis spectrophotometer (Secomam, France) at 760nm. Results were expressed as mg of gallic acid equivalents per liter (mg GAE/L) of juice. Experiments were run in quadruplicate.

2.5 Determination of Antioxidant Activity

The antioxidant properties of pomegranate-based juices were assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method [30]. All juices were diluted in ratio 1:2 with methanol: water (6:4). 100 μl of diluted juice was mixed with 2 ml of 0.1 mmol/L DPPH in methanol. After incubating at room temperature for 5 minutes in the dark, the absorbance of the mixture was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage according to the formula:

$$\% \ DPPH = (Ac - As) / Ac \qquad ((1)$$

where As and Ac were the absorbance of sample and control solutions, respectively. Each measure was replicated 3 times.

2.6 Preparation of Bacterial Strains

The strains used included *Escherichia coli* ATCC 35218 (Gram-negative bacteria), *Listeria monocytogenes* and *Staphylococcus aureus* ATCC 29213 (Gram-positive bacteria). They were maintained at -80ºC in Luria broth (LB) containing 20% glycerol. Stock cultures were maintained in Tryptic Soy Agar (TSA). Prior to the experiment, the working cultures were prepared by subculturing of each stock culture in 3 ml of Tryptic soy broth and were incubated at 37ºC for 24 h. After incubation, 100 μl of bacteria from each working culture were individually inoculated in fresh Tryptic soy broth and were incubated at 37ºC for 1 h (*E. coli*) and 1.5 h (other strains) in order to obtain inocula containing cultures in the exponential growth phase of approximately 1×10^7 cfu/ml [31].

2.7 Growth Inhibition Kinetics

Growth inhibition kinetic curves for the bacteria were performed on juices adjusted at pH 7.0. After filtration under sterile conditions through a 0.22-μm-pore-size filter, 10 ml of juices were inoculated with each bacterium to obtain a final concentration of 103 cfu/ml. Growth controls were composed of phosphate buffered saline (PBS) solutions at pH 7.0 supplemented with 14% sucrose and were inoculated with each bacterium at the final concentration of 10^3 cfu/ml. Inoculated juices and growth controls were incubated at 37ºC under agitation for 5 h, and samples were collected at 0, 60, 120 and 300 min and were plated, using the spread plate technique, on sterile Petri plates containing TSA. Plates were incubated for 24 h at 37ºC, and results were expressed as cfu/ml [31].

III. Results And Discussion

3.1 Total Phenolics and pH Values

The chemical composition of the fruits differs depending on the cultivar, growing region, climate, maturity, cultivation practice, and storage conditions [32]. Moreover the phenolic content in juices is strongly dependent on the processing steps to obtain the juice [7]. Phenolic content is varied in commercial pomegranate juices from different countries: 144 to 10086 mg GAE/L in Turkey [30] and 3429 mg GAE/L in USA [7]. Also blackberries, strawberries and red grapes are considered a rich source of phenolic compounds [33- 35]. A study showed that the phenolic content of commercial grape juices in Brazil was between 1117 and 3433 mg GAE/L [33]. Other studies reported the level of phenolic compounds of blackberry juice as 1831 and 3664 mg GAE/L and strawberry juice as 1271 mg GAE/L [7, 36]. Data on the total phenolic content and pH of commercial pomegranate-based juices that we measured are presented in Table 1. Of course, if the fruit varieties used the processing conditions during the juicing and other factors that affect the composition are not known, the only reliable conclusion can be drawn from the comparison of the juices of each company. Obtained results indicate large difference between the natural pomegranate juice and the pomegranate juice from concentrate. Specifically, the total phenolic content in the pomegranate juice from concentrate was almost 7-fold higher than that of the natural pomegranate juice. The addition of natural blackberry and strawberry juice slightly raised the total amount of phenolic compounds. It is obvious that natural blackberry and strawberry juice contain higher levels of phenolic compounds than natural pomegranate juice. On the contrary, grape juice contains less phenolic compounds than pomegranate juice from concentrate. Large fluctuations in the phenolic components of commercial pomegranate juices were found by other investigators, such as 144-10086 mg GAE / L [30], 1193-2630 mg GAE / L [37], 2566 mg GAE / L [38] and 1751-3519 mg GAE / L [39].

Table 1 Total phenolic contents and pH values of commercial pomegranate-based juices.

Sample	Total phenolics (mg GAE/L)	pH
natural pomegranate juice	720.55±1.03	3.50±0.03
natural pomegranate-blackberry juice	924.18±1.12	3.67±0.05
natural pomegranate-strawberry juice	871.46±0.80	3.72±0.05
pomegranate juice from concentrate	5158.55±0.82	3.91±0.05
pomegranate-grape juice from concentrate	4118.55±2.18	4.00±0.03

Values are mean ± standard deviation

As also seen in Table 1 a slight increase in pH was found when pomegranate juice was mixed with other fruit juices.

3.2 Antioxidant Activity

Antioxidants are substances that prevent or delay oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species as well as free radicals. The main health damaging compounds, which are scavenged by antioxidants, include reactive free radicals such as superoxide, hydroxyl, peroxyl and alkoxyl radicals and also non-radical species such as hydrogen peroxide etc. The most well known antioxidant constituents of fruits and vegetables, which may play these roles of prevention and protection, are vitamins C and E, carotenoids, minerals (selenium and zinc), some peptides and phenolic compounds. These latter are the most ubiquitous antioxidants. Several data have revealed their high antioxidant capacities and their health promoting effects [40]. Earlier studies showed that blackberry and strawberry have a high antioxidant capacity showing high correlation with the content of phenolic compounds [34, 41]. Moreover the potent antioxidant properties of commercial pomegranate juices have been attributed to their high content of polyphenols [42]. However, the relationship between phenolic compounds and antioxidant capacity of grape extracts was inconsistent among the results from different studies [43]. Radical scavenging capacity determined by the DPPH method for analyzed samples is presented in Figure 1. The highest DPPH radical scavenging activity (%) was identified for pomegranate juice from concentrate (91.1%) followed by pomegranate-grape juice from

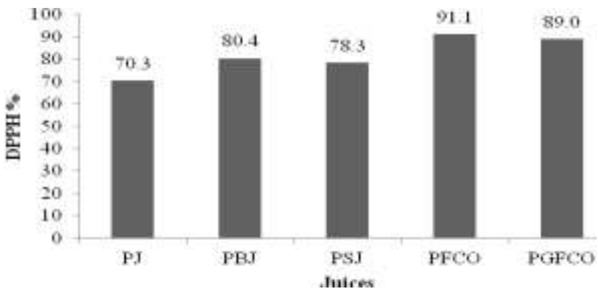

Figure 1 Antioxidant capacity determined by DPPH radical scavenging activity assay of five commercial pomegranate-based juices.

concentrate (89.0%), natural pomegranate-blackberry juice (80.4%) and natural pomegranate-strawberry juice (78.3%). Natural pomegranate juice presented the lowest value (70.3%). According to the results (Table 1 and Figure 1) the higher concentration of phenolic content of the juices is associated with higher radical scavenging activity. Studies by other researchers have shown similar effects on the antioxidant activity of pomegranate juice, such as binding of DPPH radical in an amount 63,1-73,6% [44] and 67-72% [45]. On the other hand, different results showed another study on the antioxidant activity of commercial pomegranate juice, in which the DPPH blocking rates varied between 25.19 and 67.46% [30].

3.3 Effects of Juices on Growth Inhibition Kinetics

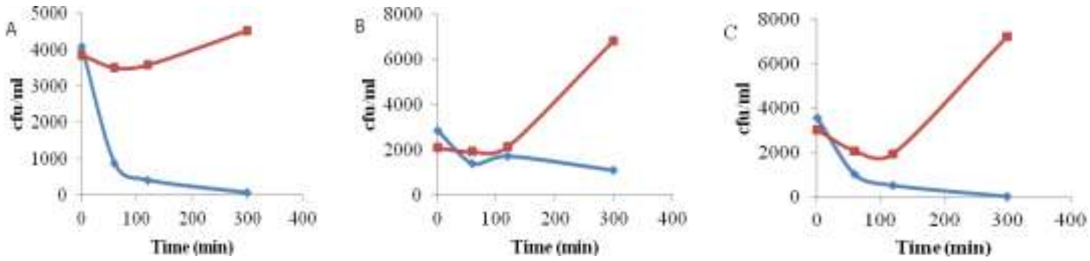

Figure 2 Growth inhibition kinetics of *S. aureus* (A), *L. monocytogenes* (B) and *E. coli* (C) in pomegranate juice from concentrate at pH 7.0 (-♦-) and in control (-■-).

The antibacterial activities of different pomegranate juices against *S. aureus*, *L. monocytogenenes* and *E. coli* are presented in Figures 2-6. The results showed that the most sensitive bacterium, among the ones tested, was *S. aureus*, because its exposure to pomegranate juice from concentrate, pomegranate-grape juice from concentrate and natural pomegranate-strawberry juice almost eliminated it (Fig. 2A, 3A and 6A). The natural pomegranate juice or the natural pomegranate-blackberry juice also inhibited the growth of *S. aureus* (Fig. 4A, 5A). In case of *L. monocytogenes*, pomegranate juice from concentrate and pomegranate-grape juice from concentrate had only moderate inhibitory activity (Fig. 2B and 3B). On the contrary, *L. monocytogenes* population was unaffected by the natural pomegranate and natural pomegranate-blackberry juice (Fig. 4B and 5B). Finally, despite the fact that the *E. coli* population was significantly reduced in most juices, natural pomegranate-blackberry juice was the only juice that did not affect its growth (Fig. 5C). However, further research would be needed to confirm this finding.

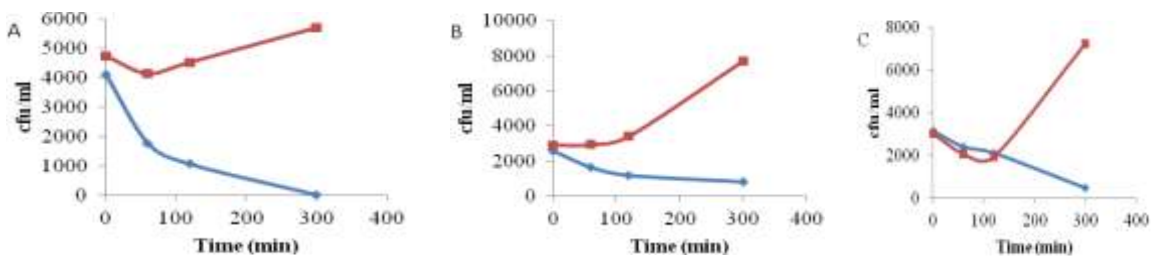

Figure 3 Growth inhibition kinetics of *S. aureus* (A), *L. monocytogenes* (B) and *E. coli* (C) in pomegranate-grape juice from concentrate at pH 7.0 (-♦-) and in control (-■-).

The presence of common phytochemicals in the plant extracts including phenols, tannins, and flavonoids as major active constituents may be responsible for antimicrobial activities [46, 47]. Phenolics attack the cell wall of the microbes and penetrate the cell, where they react with the cytoplasm and the cellular proteins [48]. Probable targets on the microbial cell are surface exposed adhesion molecules, cell wall polypeptides and membrane-bound enzymes. The mechanisms thought to be responsible for the phenolic toxicity towards microorganisms, include enzyme inhibition by the oxidized compounds, possibly through reaction with sulphydryl groups or through more nonspecific interactions with proteins often leading to inactivation of the protein and loss of function [49].

Overall, broad-spectrum antimicrobial activity has been observed in pomegranate. Fractions of ellagic acid, gallagic acid, punicallins, and punicalagins extracted from pomegranate revealed antimicrobial activity when assayed against *E coli*, *Pseudomonas aeruginosa*, methicillin-resistant *S. aureus*, and other harmful bacteria [3]. There are reports showing the antimicrobial activity of pomegranate juice concentrate against *S. aureus*, *P. aeruginosa*, *Klebsiella pneumoniae*, *E. coli*, *Bacillus cereus*, *Bacillus coagulans*, *Bacilus subtilis*, as well as its antifungal effect to *Aspergillus niger*, *Mucor indicus*, *Penicilium citrinum* etc [50]. Another study revealed that pomegranate juice concentrate was found active against both Gram-positive and Gram-negative bacterial species. However, the activity against Gram-positive organisms was higher compared to Gram-negative species [19].

The relative resistance of Gram-negative bacteria compared to Gram-positive bacteria resides to the outer membrane of Gram-negative bacteria acting as a permeability barrier, so that the uptake of the compounds in the cell is reduced [49]. Our results are in general agreement with these findings. Nevertheless, the antibacterial action of pomegranate juice varies with variety and depends on the contents of phenolic compounds, pigments and citric acid [50].

Berry fruits have also antimicrobial activities. For example, commercial dark red grape juice from concentrate showed antilisterial activity but no inhibitory action against *E. coli* and S. aureus [51]. Furthermore, blackberry juice had better antibacterial activity against *S. aureus* and less against *E. coli* [52]. At this point it should be emphasized that sometimes comparison between different studies and their results is difficult, since different authors have used different experimental settings, or the used methods are only partially reported.

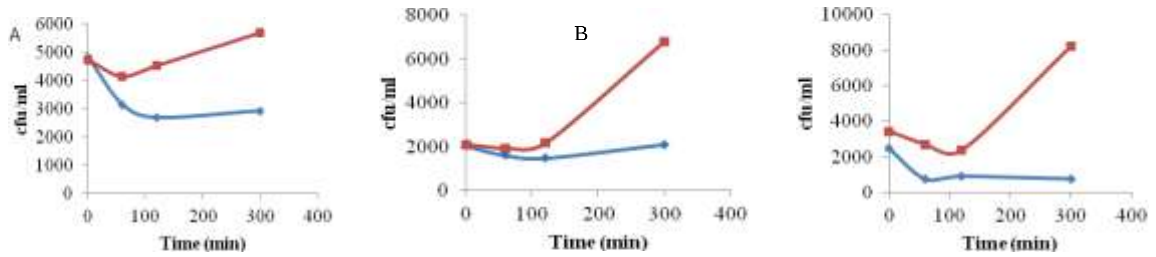

Figure 4 Growth inhibition kinetics of *S. aureus* (A), *L. monocytogenes* (B) and *E. coli* (C) in natural pomegranate juice at pH 7.0 (-♦-) and in control (-■-).

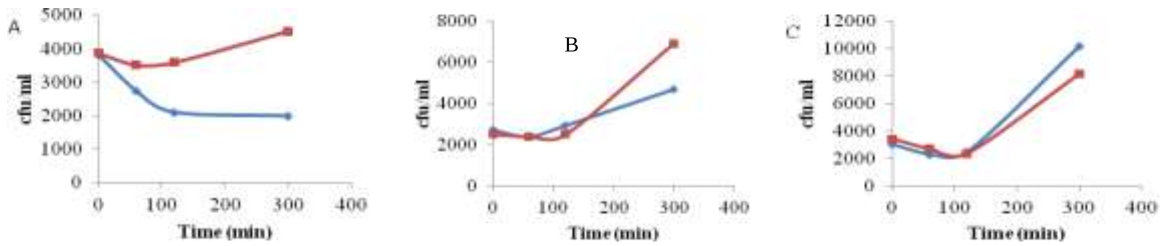

Figure 5 Growth inhibition kinetics of *S. aureus* (A), *L. monocytogenes* (B) and *E. coli* (C) in natural pomegranate-blackberry juice at pH 7.0 (-♦-) and in control (-■-).

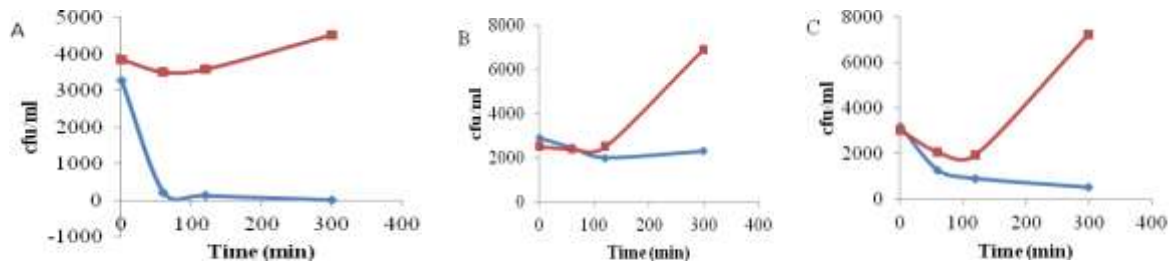

Figure 6 Growth inhibition kinetics of *S. aureus* (A), *L. monocytogenes* (B) and *E. coli* (C) in natural pomegranate-strawberry juice at pH 7.0 (-♦-) and in control (-■-).

IV. Conclusions

Considering the importance of functional food and beverages, results of this study demonstrate that the commercial pomegranate based juices have strong antioxidant activity. In addition, juices with higher phenolics concentration resulted in elimination of both Gram-negative (*E. coli*) and Gram-positive bacteria (*S. aureus*). Finally, among all pomegranate based juices, pomegranate juice from concentrate and pomegranate-grape juice from concentrate might be the best option according to health benefits.

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