





Article

Effects of the Extraction Technology on Pomegranate Juice Quality

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Abstract: Pomegranate juice is a rich source of phenolic components; its consumption has considerably increased throughout the world in recent years, due to its nutraceutical properties. Commercial juice production involves pressing the fruits. The aim of this study was to assess the influence of the pressing stage on pomegranate juice properties, in terms of value, duration of the applied pressure and juice yield in order to examine the influence of pressure level on volatiles and nutraceutical properties of the juice. Pomegranate fruits cv. Wonderful One were manually harvested and mechanically processed for extracting the juice by means of a shelling machine, a peristaltic pump and a pneumatic press. Chemical analytical determinations were performed on the juice samples corresponding to the different pressure levels applied. They did not show a univocal trend with respect to the increase in pressure; total phenol content values gradually increased as the pressure applied increased, conversely the highest total anthocyanins value was obtained in the first step of the process (552 mg L⁻¹), afterwards a 40% decrease occurred. More than forty Volatile Organic Compounds were identified in the obtained pomegranate juices. The results showed a significant increase in the values in some compounds, particularly for pressure values higher than 0.7 bar, while in others there was a significant decrease as pressure increases. Therefore, the application of different pressure values during pomegranate juice extraction process allowed to obtain products of different quality.

Keywords: polyphenols; anthocyanins; antioxidant activity; fruit processing; mechanical extraction; pneumatic press

1. Introduction

Pomegranate tree (*Punica granatum* L.), native of Southern Asia, belongs to the family of Punicaceae. This species is widely grown in Iran, India, Egypt, Lebanon, China, Spain, France, USA, Oman, Syria, Tunisia, Italy, Greece, Cyprus, Israel, Turkey, Chile, Portugal and South Africa [1,2].

The fruit is a spherical berry, having a very consistent peel and divided into loggias, hosting grains called arils and wrapped in a garnet-red juicy pulp [3]. It is commonly consumed fresh or as juice. In addition, the fruit is used in food industry for manufacturing jellies and concentrates, as well as flavouring and colouring agents.

Pomegranate juice has many health properties, as it contains anticarcinogenic, antimicrobial and antiviral compounds. In fact, pomegranate fruit is a rich source of bioactive compounds, i.e., flavonoids, phenolic acids and vitamin C, mainly found in the peel, pith and arils and, therefore, in its juice [4]. The pomegranate juice is a rich source of antioxidants, that were found to be higher than in other natural beverages, i.e., green tea and red wine.

The stability and concentration of these functional properties are affected by pre-harvest factors, i.e., cultivar, agro-climatic conditions, maturity status, harvest season, irrigation and fertilisation, and post-harvest factors, i.e., storage, packaging and treatments [5].

Several studies have documented the benefits of pomegranate juice consumption in individuals affected by various disorders [6,7]. In fact, a number of scientific studies have demonstrated that the consumption of pomegranate fruit or juice confers medicinal benefits in the prevention of cardiovascular diseases, diabetes and cancer [8,9].

Therefore, its consumption has highly increased throughout the world in recent years, due to the potential of its components and, above all, polyphenols and anthocyanins [10]. Some studies have proved the antioxidant [11] and anti-inflammatory [12] activity of the phenolics included in the pomegranate juice.

Many studies have demonstrated the influence of the extraction technology and machines on the organoleptic and physical-chemical properties of the juice. Fischer et al. [13] obtained juice by means of a pilot plant from steamed and peeled pomegranate fruits, respectively, by applying increasing pressure and various juice treatments (enzymatic treatment, filtration, clarification and pasteurisation). The total amounts of anthocyanins and colourless phenolics in the juice significantly differed depending on the applied technology. In Fischer et al. [14] a more comprehensive knowledge of fruit composition and its impact on pomegranate juice features were performed on both fresh pomegranates and juices. Türkyılmaz et al. [15] produced pomegranate juice samples with three different pressing programs: 1.2–4.8 bar for 25 min; 1.2–2.4 bar for 15 min; 1.2–1.8 bar for 5.5 min. Respective juice yields were 39.2%, 33.2% and 27.2%. Koppel et al. [16], in order to determine the effect of the extraction technology on pomegranate juice flavour characteristics, aromatic compounds and physical-chemical properties, made fresh, fresh frozen and pasteurised, as well as dried and reconstituted juice samples from Wonderful cv. fruits. The extraction technology had an effect on pomegranate juice properties but this effect was different depending on the selected technology.

Above all, Mphahlele et al. [17] investigated the chemical, volatile composition and bioactive compounds extracted by means of different methods of extraction of pomegranate, cv. Wonderful (i.e., the same cultivar surveyed in this work). The results indicated that the extraction technology significantly influenced pH, titratable acidity, yield and colour of pomegranate juice and, therefore, its quality.

In a previous study [18] the authors evaluated the effects of two different plants, one was constituted by a hydraulic press and the other comprised a pneumatic one, on pomegranate juice yield and health properties: they obtained a 15% higher yield by using the pneumatic press. Furthermore, the pomegranate juice obtained by using the pneumatic press showed a higher nutraceutical value, especially described by free radical-scavenging activity and titratable acidity.

The aim of this study is to assess the influence of the pressing stage on pomegranate juice properties, in terms of value, duration of the applied pressure and juice yield. This in order to examine the influence of pressure level on volatiles and nutraceutical properties of pomegranate juice.

2. Materials and Methods

2.1. Material

Pomegranate fruits cv. Wonderful One were manually harvested in November 2018 in an orchard located in the province of Trapani (Italy). The plants, aged 7 years, are arranged in the field at a distance of 5 m between the rows and 3 m in the row. The training system is bush-shaped with a supporting structure consisting of galvanized iron poles and triple galvanized wires resistant to oxidation (Figure 1).

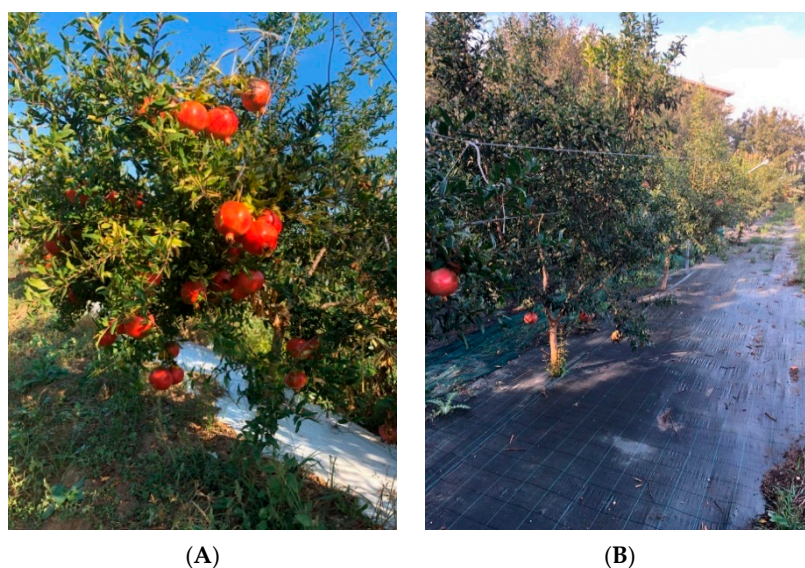


Figure 1. Bush-grown pomegranate plants (A), arranged on rows with mulched and trunked soil (B).

The 0.45 m height of the trunk from the ground and the bush growing system allow to obtain fruits of excellent quality [2]. The fruits were manually harvested and delicately placed in 25 kg bins, then they were transferred to the processing plant for extracting juice within 24 h from harvesting. Before juice extraction, pomegranate fruits were washed with cold tap water and dried. The averaged fruit weight ranged from 350 to 750 g.

Chemical analyses of pomegranate juice were performed on three samples for each case study within a week from extraction by using conventional analytical grade salt and solvent reagents during procedures described in Section 2.5.

2.2. Experimental Plant for Fruit Processing

The continuous cycle plant used for fruit processing during the tests was located inside Migel srl, a food industry in the province of Palermo, Italy; it is mainly constituted by a shelling machine (model VEGA-80, 13.8 kW), a peristaltic pump (model PRS 18, 4.0 kW, 1075 rpm, 30–180 hL/h), and a pneumatic press (model SF22) as shown in Figure 2. All the machines were manufactured by Puleo srl, Marsala, Italy. The pomegranate juice produced was addressed to the market for fresh consumption.

The fruits transported inside pierced bins are discharged in a tank, from which a sloped conveyor belt moves them inside a shelling machine. This machine is constituted by a pierced cylinder having a diameter of 0.80 m, inside which shelling winders are oriented along a spiral on a shaft. The cylinder and the shaft have an opposite rotation speed of 30 and 300 rpm, respectively. The fruits are hit by the winders, so that they are opened and shelled. Therefore, the arils are projected on the side surface of the cylinder and cross its holes (diameter 18/22 mm), while the rest of the fruit, i.e., peel and pulp, is thrown out through the rotation of winders. The arils and part of the extracted juice are collected inside a tank, placed at the bottom of the machine, from which, through a peristaltic pump, are moved to a pneumatic press. This pump has a stainless steel AISI 304 structure and an internal pipe, built of food rubber and suitable for liquid and semi-dense products. The pump is equipped with a frequency regulator, allowing to set up the flow rate. Figure 3 shows the main constituent parts of the SF22 Drain Membrane Press used for extracting the juice from the arils.

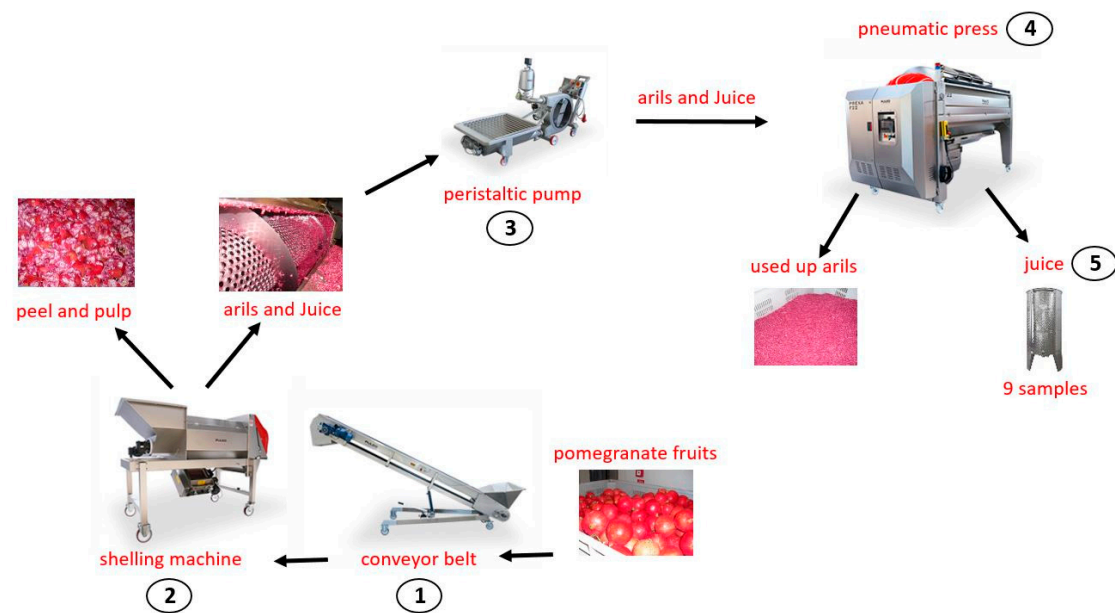


Figure 2. Continuous cycle plant used for fruit processing from pomegranate fruits unloading to juice extraction.

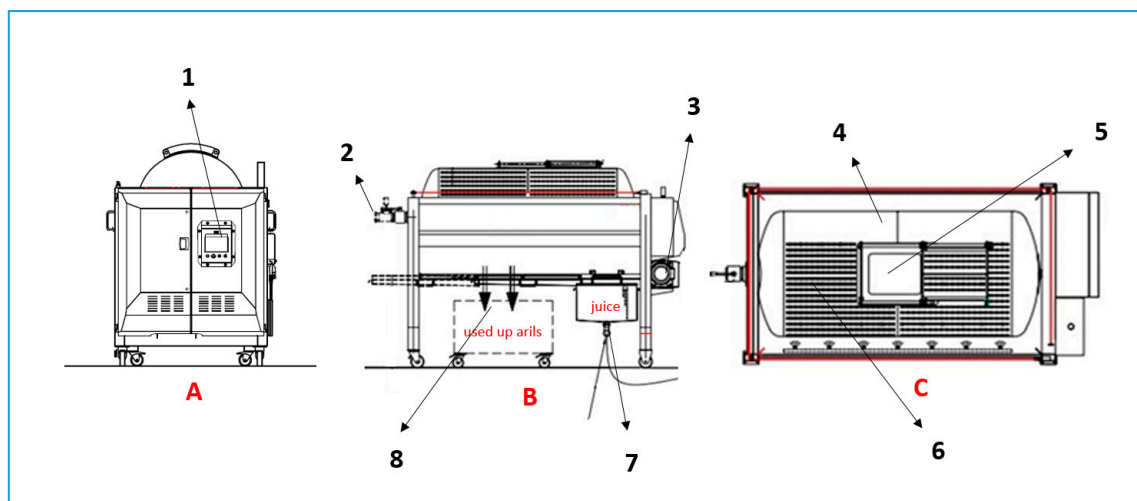


Figure 3. Drain Membrane SF22 pneumatic press used during the tests: (A) front view; (B) side view (C) plan view. (1) control panel, (2) axial loading, (3) gearbox, (4) closed cylinder, (5) shutter slides, (6) perforated cylinder (7) stainless steel tank, (8) used up arils unloading.

This pneumatic press, requiring no minimum load, is constituted by a stainless steel crankcase, that covers a stainless steel tank, a door, a frame, a heavy-duty membrane and an axial feed valve. The press has a manual or automatic operation, 12 programmable cycles, 12 steps to be set up within each cycle and an on board compressor. The side surface of the press cylinder has a perforated part and a closed one (Figure 4).

A membrane, placed in the closed part, is inflated by the air produced by the compressor and, therefore, pushes the arils towards the perforated part of the cylinder, by extracting the pomegranate juice. The juice comes out through the holes and is conveyed inside a stainless steel tank, placed at the bottom of the cylinder. After juice extraction by pressure, the used up arils are thrown out through the opening of two shutter slides, placed on the pierced part of the cylinder.

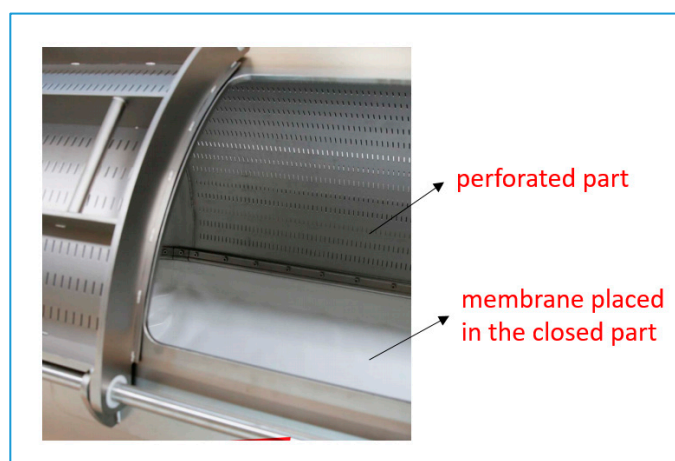


Figure 4. Cylinder inside view with the membrane which, by inflating, presses the arils on the perforated part from which the pomegranate juice comes out.

2.3. Tests

The total amount of processed pomegranate fruits was 5 t per replication; the tests were replicated three times. The total processing time was 195 min and the press working capacity was 1.5 t h^{-1} . The press feeding duration was 110 min, i.e., 56% of the total processing time. The press was programmed in order that, during feeding, the cylinder could accomplish a whole rotation every 40 s: the arils were crashed and the juice was drained in the collection tank.

The different steps of the processing cycle are reported in Table 1, corresponding to the tests used in the experimentation and the samples that were taken to be analysed. Sample T1 derives from the mechanical separation of all the components of the fruit (peel, pulp, arils) and was obtained without the application of any pressure. Juice samples from T2 to T9 were obtained only from the arils.

Table 1. Tests used in the experimentation.

Test	Pressure (bar)
T1	0 (after drainage)
T2	0 (after setting)
T3	0.3
T4	0.5
T5	0.7
T6	1.2
T7	1.4
T8	1.6
T9	1.8

The pressure of the machine was set up at 0.3, 0.5, 0.7, 1.2, 1.4, 1.6 and 1.8 bar, during seven processing cycles, respectively. The first two values of pressure were replicated twice. After every processing cycle the cylinder accomplished one or two clockwise rotations and one or two counterclockwise rotations. The diagram of the processing cycle is shown in Figure 5.

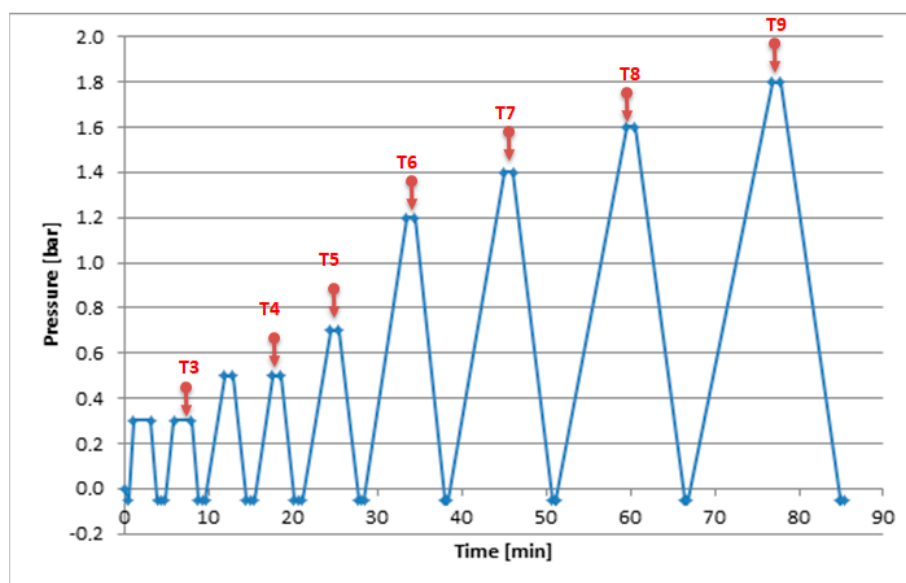


Figure 5. Diagram of the processing cycle carried out by means of the press. The arrows indicate the sampling points of juice samples.

Pomegranate juice samples were collected after each test, placed in 100 mL dark glass bottles, stored at 12 °C and transported to the laboratory, where analyses were performed.

2.4. Yield Computation

The yield of extracted Pomegranate Juice—PJ (%) was calculated by using the following equation:

$$\text{Juice yield (\%)} = \frac{\text{Weight of PJ}}{\text{Weight of whole pomegranates}} \times 100 \quad (1)$$

2.5. Chemical Analytical Determinations

2.5.1. pH

The homogenised samples of pomegranate juice were used in order to determine the pH values by means of a pH meter (model MP 220, Mettler Toledo, Columbus, OH, USA). The sample temperature was standardised at 25 °C.

2.5.2. Total Soluble Solids (TSS)

Homogenised and paper filtered samples of pomegranate juice were used for the determination of Total Soluble Solids (TSS) (°Brix) by means of an Optical Refractometer (N-50 E Hand Refractometer, ATAGO, Tokyo, Japan).

2.5.3. Titratable Acidity (TA)

6 g of juice were placed inside a 100 mL beaker, where with 50 mL of water were added. Titration were performed by using 0.1 N NaOH to an end point of 8.2 (measured by means of the pH meter), therefore the volume of the NaOH solution (mL) were recorded. Titratable Acidity (TA) was expressed as g of citric acid per 100 g of juice by using the following equation:

$$\text{citric acid (\%)} = (\text{mL NaOH used}) \times (0.1 \text{ N NaOH}) \times (\text{Mill equivalent factor}) \times (100)/\text{g of sample} \quad (2)$$

where the Mill equivalent factor of citric acid is 0.064.

2.5.4. Total Phenolic Content (TPC)

10 mL of pure ethanol were added to 2 g of each homogenised sample. The extraction was done by means of a vortex mixer (model RX3 by VELP Scientifica Srl, Usmate, Italy) for 60 s. The mixture was filtered and the filtrate was placed inside a test tube. The Folin-Ciocalteu micro method of Waterhouse was used to determine the Total Phenolic Content (TPC) [19]. 60 µL of the filtrate were diluted in 4.8 mL of Milli-Q grade water, as well as 300 µL of Folin-Ciocalteu reagent were added and shaken. After 8 min, 900 µL of 20% sodium carbonate solution were added and mixed. After reaction at 40 °C for 30 min, the absorbance was measured at 765 nm by means of the UV mini-1240 spectrophotometer (Shimadzu, Sydney, Australia). A calibration curve of gallic acid (3,4,5-trihydroxybenzoic acid) was prepared (0–50 µg) and used as standard ($R^2 = 0.98$). The results were given as mg of gallic acid equivalent per g of fresh weight.

2.5.5. Radical-Scavenging Activity (RSA) of 1,1-diphenyl-2-picrylhydrazyl Radical (DPPH)

The antioxidant activity of juice samples was measured in terms of their Radical-Scavenging Activity (RSA), by using the DPPH (1,1-diphenyl-2-picrylhydrazyl radical) method [19,20]. Aliquots of the whole pomegranate juice were mixed with an ethanol solution of DPPH (3 mM), i.e., 2.37 mg of DPPH in 2 mL of ethanol. For the sample solution, 28 µL of whole juice were mixed with 28 µL of DPPH solution and 944 µL of ethanol. After incubation in the dark at room temperature for 10 min, the spectrophotometric determination was assayed at 515 nm by means of a spectrophotometer (UV5, Mettler Toledo, Columbus, OH, USA). A freshly prepared DPPH blank solution (containing 972 µL of ethanol and 28 µL of DPPH solution) was used. The DPPH solution was stored in a flask covered with aluminium foil and kept in the dark at 4 °C from one measurement to another. The percentage decrease of absorbance was recorded for each sample and the percentage quenching of DPPH radical was calculated on the basis of the observed decrease of absorbance according to the following equation:

$$\text{Antioxidant power inhibition (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (3)$$

where A_0 is the absorbance value of the DPPH blank solution and A_1 is the absorbance value of the sample solution.

2.5.6. Total Anthocyanins (T ANT)

An aliquot of sample was diluted with a buffered solution of pH 1 (125 mL of 0.2 M KCl and 375 mL of HCl 0.2 M). A second aliquot was diluted with a buffered solution of pH 4.5 (400 mL of 1 M $\text{CH}_3\text{CO}_2\text{Na}$, 240 mL of 1 M HCl and 360 mL of H_2O). The absorbance (Abs) of the solutions was measured at 510 nm and the concentration of total anthocyanins was expressed in terms of cyanidin-3-glucoside according to the following equation:

$$C \text{ mg/L} = (\text{Abs pH 1} - \text{Abs pH 4.5}) \times 484.82 \times 1000/24,825 \times \text{DF} \quad (4)$$

where DF is the dilution factor.

2.5.7. SPME-GC-MS Analyses

Gas Chromatography–Mass Spectrometry (GC-MS) analyses were performed using a 5890 GC system (Hewlett-Packard, Palo Alto, CA, USA) interfaced with an HP 5973 quadrupole mass spectrometer detector (Hewlett-Packard, Palo Alto, CA, USA). As a stationary phase an HP5-MS capillary column (5% diphenyl—95% dimethylpolysiloxane 30 m—0.2 mm, 0.25 µm film thickness, J&W Scientific, USA) was used. Injector and detector temperatures were 250 °C and 270 °C respectively. Helium was used as the carrier gas. The GC oven temperature program was 40 °C for 5.00 min, then increased by 10 °C/min to 280 °C. Electron impact ionization spectra were obtained at 70 eV, recording mass spectra from 42 to 550 *uma*.

Compound identification was carried out using a commercial NIST 07 mass spectra library search and by comparison with standard analytical grade compounds purchased from Sigma-Aldrich (St. Louis, MO, USA). For this analysis the pomegranate juice elutes from different pressure were collected and the volatile organic fractions were extracted by solid-phase microextraction (SPME) with a polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 mm) coated fiber purchased from Supelco, Sigma-Aldrich (Bellefonte, PA, USA). The extraction was carried out after having mixed 3 g of pomegranate juice with 3 mL of saturated NaCl solution in a 15 mL glass headspace vial with PTFE/Silicone septa (Supelco, Sigma-Aldrich, Bellefonte, PA, USA) and a magnetic stirring bar. The vial was gently heated and magnetically stirred for 20 min at 60 °C to allow equilibrium. Then, the SPME fiber was inserted through the septum and exposed to the sample for 30 min. The fiber was then retracted and introduced on the GC injector and held for 3 min to allow the desorption of the analytes in the GC inlet port. Prior to use, the PDMS fibres were conditioned at 250 °C for 30 min. The linear retention indices were calculated with references to n alkanes (C6–C22), obtained from FLUKA (Sigma-Aldrich, Bellefonte, PA, USA), and run under the chromatographic condition described above. Standard mixtures of selected compounds for each different class were also injected in the GC inlet port and retention indices determined. 1 as superscript numbers near the compound name identifies the verified authentic standard molecules (as shown in the table reporting the GC-MS analyses results). Response factors of reference compounds from different classes of monoterpenes, sesquiterpenes, alcohols, esters, aldehydes, ketones and phenolic compounds were determined and found to range from 0.85 to 1.2 versus n hexanol, averaging 1.0. Response factors were therefore taken as 1.0 for all compounds and on the base of integrated area of each analytical chromatographic peak, data has been presented as percentage of the total area.

2.6. Statistical Analysis

Chemical analyses of pomegranate juice were performed on three samples for each case study within a week from extraction. The data were subjected to ANOVA and Tukey's test for mean comparison at 95% confidence level (Statgraphics Centurion, Statpoint Inc., The Plains, VA, USA, 2005).

3. Results and Discussion

Figure 6 shows that the arils obtained at the end of the process for juice extraction are headless and free of liquid. The processing line allowed to extract all the juice content of the fruit.

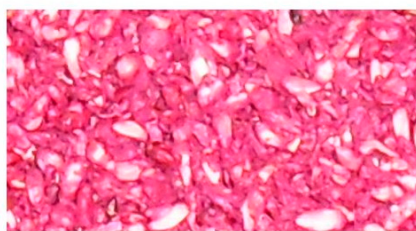


Figure 6. Arils after pressing performed with the F22 pneumatic press by Puleo Srl, Marsala (Italy).

The yield of extracted pomegranate juice resulted $33.5\% \pm 2.0$. The yield values were similar to those obtained by Türkylmaz et al., i.e., 39.2% [15], while in Fischer et al. [14], it ranged from 42.9 to 61.4%. The total yield of 33.5% was distributed as follows: 8.4% in T1, 3.3% in T2, 5.3% in T3, 4.0% in T4, 2.4% in T5, 2.7% in tests T6, T7 and T8 and 2.0% in T9 revealing a progressively lower amount of extracted water during the process.

Table 2 shows pH, Total Soluble Solids (TSS), Titratable Acidity (TA), Total Phenolic Content (TPC), Radical-Scavenging Activity (RSA) and Total Anthocyanins (T ANT) values of pomegranate juice samples obtained with different pressures of the pneumatic press during the tests.

Table 2. pH, Total Soluble Solids (TSS), Titratable Acidity (TA), Total Phenolic Content (TPC), Radical-Scavenging Activity (RSA) and Total Anthocyanins (T ANT) values of pomegranate juice samples obtained with different pressures of the pneumatic press during the tests from T1 to T9.

Test	pH	TSS (°Brix)	TA (%)	TPC (mg g ⁻¹)	RSA (%)	T ANT (mg L ⁻¹)
T1	3.04 ± 0.03 c	15.2 ± 0.2 b	1.86 ± 0.03 a	694 ± 22 f	51.6 ± 4.4 b	552 ± 8 a
T2	3.10 ± 0.02 bc	13.0 ± 0.2 e	1.58 ± 0.03 e	825 ± 21 e	64.2 ± 9.3 ab	298 ± 11 c
T3	3.14 ± 0.02 ab	14.0 ± 0.2 d	1.66 ± 0.03 cd	867 ± 20 de	71.1 ± 11.7 ab	390 ± 10 b
T4	3.08 ± 0.03 bc	14.0 ± 0.1 d	1.64 ± 0.02 de	872 ± 22 de	73.0 ± 9.4 ab	297 ± 110 c
T5	3.12 ± 0.03 ab	14.5 ± 0.1 c	1.73 ± 0.02 b	877 ± 22 de	76.4 ± 8.2 ab	302 ± 8 c
T6	3.15 ± 0.04 ab	14.0 ± 0.1 d	1.66 ± 0.03 cd	928 ± 30 bcd	77.4 ± 4.6 a	274 ± 11 c
T7	3.15 ± 0.01 ab	14.0 ± 0.2 cd	1.65 ± 0.03 cd	952 ± 25 bc	78.5 ± 8.0 a	281 ± 11 c
T8	3.10 ± 0.03 bc	17.0 ± 0.2 a	1.81 ± 0.01 a	990 ± 30 b	80.2 ± 9.0 a	368 ± 12 b
T9	3.18 ± 0.03 a	14.0 ± 0.2 cd	1.62 ± 0.02 de	1076 ± 17 a	83.2 ± 9.3 a	290 ± 10 c

Note: Data are the mean values of three independent experiments ± standard deviation. Values in each column having different letters are significantly different from one another at $p < 0.05$.

The qualitative parameters of the juice are subject to natural fluctuations caused by the variety, the ripening index, the geographical site and climatic conditions of cultivation and conservation of the fruits before their processing [21,22]. Table 2 shows the main quality parameters of pomegranate juice in the various tests; pH remains almost stable with values between 3.04 and 3.18. It resulted similar to that measured from the juice of the same cultivar by Koppel et al., ranging from 3.41 to 3.86 [16] and by Mphahlele et al., ranging from 1.85 to 3.23 [17].

The TSS values are around 14.0 °Brix in all the tests except T1 and T8 (15.2 and 17.0 °Brix respectively). The averaged value resulted similar to that measured by Koppel et al., ranging from 14.3 to 15 °Brix [16], and slightly lower than that obtained by Mphahlele et al., ranging from 16.03 to 16.34 °Brix [17], both from the juice of the same cultivar surveyed in this work.

The value of total acidity (titratable acidity) in fruit juices depends on the organic acids of the fruit. The TA values are between 1.58% and 1.86% in the different tests. The averaged value (1.72%) resulted similar to that measured by Mphahlele et al., ranging from 1.53 to 1.78%, from the juice of the same cultivar (i.e., Wonderful One) [17]. pH, TSS and TA values are also similar to those obtained by Ferrara et al. [23].

TPC values obtained are gradually increasing from T1 (694 mg g⁻¹) to T9 (1076 mg g⁻¹) with statistically significant differences. They exclusively refer to the juice, because the plant used has eliminated the skins by means of the shelling machine.

Some results obtained by other authors, on the other hand, show a higher TPC content since their pressing phase also takes into consideration the peel of the fruit which contains a high polyphenols content. Indeed, Gözlekçi et al. [24] studied four pomegranate cultivars obtaining from the skin TPC values from 1775.4 to 3547.8 mg g⁻¹ while in juice and arils they found values from 784.4 to 1551.5 mg g⁻¹ and 117.0 to 177.4 mg g⁻¹ respectively.

As for RSA values, there is an increase of about 23% from T1 to T9, with an average value of 72.94%. The averaged value resulted much higher than that measured by Negro et al. [12], ranging from 17.2 to 39.1%, from the pomegranate juice of four different ecotypes.

Finally, the highest T ANT value is obtained in T1 (552 mg L⁻¹) while in the other tests there is approximately a 40% decrease. Anthocyanins are present in considerable quantities in the skins [25–27], and this justifies the higher value of T ANT in T1 and the subsequent decrease in the following steps in which pressure is applied in order to extract the juice only from the arils. The average value extracted from the above juice resulted similar than that measured by Gil et al. from the commercial pomegranate juice, i.e., 387.4 mg L⁻¹ [11]. Overall our results are in agreement with those obtained by Türkylmaz et al. 2013 [15]. As the pressure and time increased, TPC also increased; as pressure and time increased, T ANT decreased.

The chromatograms allowed an immediate comparison of the extracted compounds profile. Significant differences were found considering the qualitative composition of the juice volatile fraction at 60 °C and the relative quantitative amount of the identified compounds. In accord with previously declared intent, Table 3 reports, according to their increasing Retention Index (RI), the identified components together with the correspondent percent amount obtained as the medium value of 6 different chromatographic analyses.

More than forty VOCs were identified in experimental pomegranate juices. The identified VOCs in the present study suggested that such differences in our data might be related to the method of pressing the fruits [18]. 17 alcohols, 10 terpenes, 6 esters, 6 aldehydes, 4 ketones, 3 carboxylic acid, and 6 phenolic compounds were identified. Moreover the concentration of aldehyde compounds is formally related with the extraction procedure showing the increase of concentration with an increase of pressure extraction. Several phenolic compounds were identified by GC-MS method with quantitative differences depending on the extraction pressure. Moreover considering the total amount of TPC, reported in Table 2, the quantitative differences of the identified compounds are not consistent in order to determine any general conclusion about the technological procedures of extraction, in fact each compound showed certain specificity. The main alcohol and also VOCs was hexanol, with a relative abundance between 44 and 26%. In particular, from T1 to T9 3-methyl-2-buten-1-ol decreases of about 83%; its value, in fact, goes from 14.5 in tests 1, 2 and 3 to 2.35 in tests 8 and 9. The compounds (Z) 3-hexen-1-ol and α -terpineol, on the other hand, show an increase of about 65% respectively from tests 1, 2, 3, 4 and 5 to test 9 and 68% from tests 1, 2, 3 and 4 to test 9. Terpenes, one of the most extensive and varied structural compounds occurring in nature, display a wide range of biological and pharmacological activities. They are considered like the most promising strategy to prevent oxidative damage determined by Reactive Oxygen Species that are involved in the pathological development of many important human diseases such as neurodegenerative diseases, cardiovascular processes and diabetes [7–9]. Considering the *p* values of the relative abundance of limonene, also reported in Table 3, and the relative abundances of other terpenes (E)- α -bergamotene, (E)-caryophyllene, (E)- β -farnesene the higher relative total amount of these antioxidant molecules was revealed at a pressure of 1.4 bar with a 25% of total VOCs. β -myrcene undergoes a strong decrease of about 75% in tests from T7 to T9 with pressures higher than 1.4 bar, compared to the initial tests (from T1 to T6) with pressures lower than 1.2 bar. As for esters, the highest values of methyl dihydrojasmonate were obtained up to T3; as pressure increases, it reduces by about 33% in T5 and 67% in T9. Moreover, in the aldehydes group there is an increase in hexanal and phenylacetaldehyde when the operating pressures increase which occurs starting from T5, when the pressure overcomes 0.7 bar. In the phenolic compounds, there is a styrene reduction with the exceeding of 0.5 bar and a guaiacol increase in T9 at the end of the process (pressure equal to 1.8 bar).

Table 3. Mean contents (relative percentages) of the Volatile Organic Compounds (VOCs).

Compounds	RT	RI	T1	T2	T3	T4	T5	T6	T7	T8	T9	P
Alcohols (17)												
Etanol	5.09	843.86	0.3 d	0.4 d	1.2 c	1.9 a	n.d	n.d	0.7 d	1.6 b	nd	**
2-Methyl	6.96	976.40	nd	nd	nd	n.d	nd	nd	nd	0.98	nd	-
3-Methyl	11.42	1171.33	nd	nd	nd	nd	nd	nd	nd	1.6	nd	-
(E)-2-Hexen-1-ol ¹	12.03	1191.67	0.2 b	0.3 b	0.6 b	0.6 b	0.8 b	0.6 b	2.4 a	nd	nd	*
3-Methyl-2-buten-1-ol	14.55	1297.84	14.7 b	14.5 b	14.9 b	15.1 a	13.1 c	12.7 c	6.8 d	2.4 e	2.3 e	***
1-Hexanol ¹	15.51	1345.73	33 b	34 b	32 b	31 b	26 c	27 c	29 b	32 b	44 a	**
(Z)-3-Hexen-1-ol	15.80	1361.81	6.1 d	6.4 d	6.6 d	6.9 d	7.2 d	8.6 c	13.0 b	15.2 b	18.0 a	**
Heptenol	16.82	1413.69	nd	nd	nd	nd	nd	nd	nd	1.1a	1.0a	NS
Hexalin(Cyclohexanol)	16.99	1423.81	nd	nd	nd	nd	nd	nd	0.5b	1.3a	0.8b	*
2-Ethyl-1-hexanol	17.35	1445.24	1.7 a	1.8 a	1.6 a	1.6 a	1.3 a	1.3 a	0.5 c	nd	1.1 b	*
L-Linalool	17.40	1448.21	nd	nd	nd	nd	nd	nd	nd	0.42 a	1.09 a	NS
4-Terpineol	18.32	1502.98	0.8 d	0.9 d	1.6 c	1.9 c	2.9 b	2.6 b	3.4 a	1.2 d	0.8 d	**
1-Nonanol ¹	19.03	1552.41	nd	nd	nd	nd	nd	nd	3.8	nd	nd	-
β-Citronellol	20.22	1638.76	nd	nd	nd	nd	1.3 a	1.0 a	nd	0.3 b	nd	***
1-Decanol	21.20	1716.24	nd	nd	nd	nd	nd	nd	nd	nd	0.97	-
α-Terpineol	24.40	2006.54	1.4 d	1.5 d	1.7 d	1.9 d	2.7 c	3.2 c	3.8 b	4.3 b	4.7 a	***
Phenylethyl alcohol	27.27	>2000	nd	nd	nd	nd	nd	nd	1.2	nd	nd	-
Terpenes (10)												
Limonene	7.84	1031.00	3.90 e	3.85 e	4.14 e	4.84 d	5.04 d	5.94 c	6.10 c	7.70 b	8.30 a	***
β-Pinene ¹	9.79	1117.00	nd	nd	nd	nd	nd	nd	1.1 a	1.2 a	nd	NS
β-Myrcene	10.31	1134.33	14.0 b	15.0 b	18.0 a	15.0 b	17.0 a	15.0 b	3.4 d	3.5 d	5.1 c	***
γ-Terpinene	12.68	1217.24	nd	nd	nd	nd	nd	nd	0.8	nd	nd	-
p-Cymene ¹	13.41	1248.71	nd	nd	nd	nd	nd	nd	1.4	nd	nd	-
α-Pinene	16.24	1382.41	nd	nd	nd	nd	2.4 a	2.4 a	nd	nd	nd	NS
α-Terpinolene	18.25	1498.81	1.2 a	1.3 a	1.1 a	1.0 a	0.8 b	0.8 b	nd	nd	nd	*
(E)-α-Bergamotene	21.68	1757.26	nd	nd	nd	nd	0.3 b	0.4 b	3.7 a	0.3 b	nd	***
(E)-Caryophyllene	22.16	1798.29	2.6 b	2.8 b	2.7 b	2.9 b	2.8 b	2.7 b	5.7 a	2.5 b	2.0 c	**
(E)-β-Farnesene	24.11	1978.85	nd	nd	nd	nd	nd	nd	2.8 a	0.1 b	nd	***

Table 3. Mean contents (relative percentages) of the Volatile Organic Compounds (VOCs).

Compounds	RT	RI	T1	T2	T3	T4	T5	T6	T7	T8	T9	P
Esters (6)												
Ethyl acetate ¹	4.39	744.44	nd	nd	nd	nd	nd	nd	1.1 b	14.0 a	nd	***
Hexyl isobutyrate	21.22	1717.95	nd	nd	nd	nd	nd	nd	2.3 a	0.3 b	nd	***
3-Methyl butyl acetate	21.84	1770.94	nd	nd	nd	nd	nd	nd	nd	1.91	nd	-
Methyl dihydrojasmonate	22.57	1835.14	3.4 a	3.2 a	3.3 a	2.9 b	2.3 c	2.1 c	1.2 d	1.2 d	1.1 d	**
Ironmonocarbonyl	26.58	>2000	1.9 a	1.9 a	1.9 a	1.3 a	0.5 b	0.5 b	0.3 b	nd	nd	**
Methyl salisylate	27.22	>2000	1.8 a	1.0 a	1.7 a	1.6 a	0.6 b	0.6 b	0.5 b	nd	nd	**
Aldehydes (6)												
Hexanal ¹	8.17	1042.78	0.6 e	0.8 e	0.7 e	0.9 e	1.0 ce	1.3cd	1.5 cd	3.5 b	5.7 a	**
(E)-2-Hexen-1-al	12.20	1197.33	nd	nd	nd	nd	nd	nd	0.8 a	nd	0.8 a	NS
Nonanal ¹	16.01	1370.85	nd	nd	nd	nd	0.3a	0.3 a	0.4 a	nd	nd	NS
2,4-Hexadienal	17.01	1425.00	nd	nd	nd	nd	nd	nd	0.8 a	nd	0.8 a	NS
Benzaldehyde ¹	20.52	1662.02	nd	nd	nd	nd	nd	nd	0.6	nd	nd	-
Phenylacetaldehyde	23.40	1910.58	0.9 c	1.0 c	1.3 b	1.4 b	2.9 a	2.7 a	1.5 b	0.9 c	3.1 a	**
Ketones (4)												
6-Methyl-5-hepten-2-one	14.71	1305.53	nd	nd	nd	nd	nd	nd	nd	0.3	nd	-
2-Nonanone	16.08	1412.50	1.1 a	1.1 a	1.1 a	0.9 a	nd	nd	nd	0.3 b	0.8 a	*
p-Menth-1-en-3-one semicarbazone	16.92	1419.64	0.2 a	0.3 a	0.4 a	0.4 a	nd	nd	0.4 a	nd	0.4 a	NS
6-Methyl- γ -ionone	21.37	1730.77	nd	nd	nd	nd	nd	nd	nd	nd	0.3	-
Acids (3)												
3-Methyl butanoic acid	22.25	1806.31	0.3 b	0.2 b	0.3 b	0.4 b	nd	nd	nd	0.8 a	nd	*
Octanoic acid ¹	23.38	1908.65	nd	nd	nd	nd	nd	nd	nd	1.3	nd	-
Decanoic acid ¹	25.05	2067.29	nd	nd	nd	nd	nd	nd	1.0 b	nd	1.7 a	**
Phenolic compounds (6)												
Styrene (Cinnamane)	12.98	1230.17	3.9a	3.8 a	3.7 a	3.0 b	2.5 c	2.2 c	nd	0.7 d	0.5 d	**
Methyl(1-methylethenyl)-benzene	18.28	1500.60	nd	nd	0.30 c	0.40 c	0.60 b	0.65 b	0.80 a	0.90 a	nd	*
2,3-Dimethyl oxirane	18.46	1513.10	0.8 a	1.0 a	0.9 a	0.7 a	nd	nd	0.3 b	nd	nd	*
6-Methyl-2-phenylindole	20.57	1665.89	nd	nd	nd	nd	0.9 a	0.4 b	nd	nd	0.4 b	*
Guaiacol	25.36	2096.26	0.8 b	1.0 b	0.9 b	1.0 b	1.1 b	0.9 b	0.8 b	1.1 b	1.8 a	*
Methyl isoeugenol	38.08	>2000	nd	nd	nd	nd	2.0 a	2.1 a	1.4 c	1.0 c	1.9 b	**

Note: Mean values ($n = 6$) followed by different letters in the same row indicate significant differences ($p \leq 0.05$) for the juice or pomegranate fruits; significant difference at values $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ in the juice pomegranate fruits; nd: not detected; NS: not significant; RI: retention index based on identified compound retention times (RTs) calculated from linear equation between each pair of straight alkanes (C5–C22). ¹ Compounds verified with authentic standards. All compounds were also considered to be tentative (based on the mass spectroscopy libraries, Wiley7n.1 and Nist 07.L).

4. Conclusions

The study consisted in evaluating the quality of the pomegranate juice extracted using a processing plant with a soft press at low operating pressures in order to process perfectly intact arils from the beginning to the end of the process. The quality of the juice obtained is therefore not influenced by the compounds present in the remaining parts of the fruit (peel, mesocarp and seeds). The results obtained using the different operating pressures indicate that with the increase in pressures, some compounds are extracted in greater quantities, for example total phenol content, others instead in smaller quantities such as anthocyanins. With regard to VOCs, from the study it can be stated that increasing pressure, there is a significant increase in the values in some compounds: (Z) 3-hexen-1-ol, α -terpineol, hexanal and phenylacetaldehyde; while in others there is a significant decrease: methyl-2-buten-1-ol and β -myrcene. This suggests that the application of different pressure values during pomegranate juice extraction process allows to obtain products of different quality according to the commercial destination of the final product.

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