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The application of high hydrostatic pressure for the stabilization of functional foods: Pomegranate juice

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ABSTRACT

The paper aims at investigating the potential application of non-thermal innovative technologies, such as high hydrostatic pressure, for the sanitization of functional liquid foodstuffs. A 100% pomegranate juice was selected for the experiments, due to its high bioactive compounds content. The operating pressure, temperature and holding times at the pressure set point were changed over a wide range, with the aim of optimizing the processing condition in order to assure the microbiological stability of the processed juice as well as preserve the natural content of the functional compounds. The experiments clearly demonstrate that the high pressure treatment at room temperature improves the quality of pomegranate juice, increasing the intensity of red color of the fresh juice and preserving the content of natural anthocyanins. The residual activity of some enzymes at the end of high pressure processing, independently on the processing conditions, such as the polyphenoloxidase (PPO), causes the degradation of the nutraceutical compounds as observed in particular processing conditions, thus suggesting that the optimal combination of the processing parameters should take into account the degradation of the anthocyanins as well as the enzymatic activity.

1. Introduction

Rapid developments in science and technology, increasing healthcare costs, changes in food laws affecting label and product claims as well as increasing interest in attaining wellness through nutrition are among the main factors supporting the request for foods with clear effects on human health, generally defined "functional foods". On the other hand, scientific research indicates that there are many clinically demonstrated and potential health benefits from particular biologically active components, known as "functional compounds", due to having health benefits or desirable physiological effects. Pomegranate juice represents one of the foods recently promoted for its health benefits. For instance, a glass of pomegranate juice contains about 40% of the Recommended Daily Allowance (RDA) of Vitamin C. It also contains Vitamin A, Vitamin E and folic acid in reasonable quantities. Furthermore, pomegranate juice is an important source of anthocyanins, such as 3-glucosides and 3,5-diglucosides of delphinidin, cyanidin, and pelargonidin (Du et al., 1975). Moreover, several studies have highlighted the antioxidant and antitumoral activity of pomegranate tannins (punicacortein) and the antioxidant activity of the fermented pomegranate juice (Schubert et al., 1999). The main antioxidant compounds in pomegranate juice are hydrolysable tannins, but anthocyanins and ellagic acid derivatives also contribute to the total antioxidant capacity of the juice (Gil et al., 2000). Moreover, the antioxidant activity of the pomegranate juice can be correlated to the phenolic composition. In particular, it has been demonstrated that the consumption of this juice decreases the susceptibility of LDL (Low Density Lipoprotein) to aggregation and retention. Unfavorably, the bioactive compounds are quickly affected by exogenic factors such as oxygen, light, and especially pH and temperature. Therefore, there is a real need to minimize the degradation of the functional molecules during the pasteurization process and storage time of the pomegranate juice, in order to secure an optimal sensorial and nutritional quality. The thermal treatment of the juice and subsequent storage at room temperature represent the critical phases of the pomegranate transformation chain. Therefore, the challenge to preserve the nutraceutical properties suggests the application of non-thermal innovative technologies for the sanitation of the pomegranate juice. Among these technologies, high pressure processing (HPP) has the potential to produce high-quality foods that display characteristics of fresh products, are microbiologically safe and have an extended shelf life (Hogan et al., 2005; Patterson, 2005). Moreover, HPP has been used for processing several red-fruit based products (Rastogi et al., 2007; Talcott et al., 2003; Zabetakis et al., 2000).

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The present paper aims at analyzing the effects of pressure, temperature and processing times on the main parameters related to the quality of pomegranate juice: the color parameters, the content of tannins, anthocyanins, polyphenols, the aroma composition. A detailed analysis of the experimental data will allow the optimal combination of the processing parameters to be selected as well as verify the possible implication of HPP application on the selected juice.

2. Materials and methods

2.1. Pomegranate juice extraction

Pomegranates (*Punica granatum*) obtained from the local market, were used for the extraction of the juice. This fruit is nearly round, 2-1/2 to 5, crowned at the base by a prominent calyx. The tough, leathery skin or rind, is typically yellow overlaid with light or deep pink or rich red. The internal seeds are separated by a membranous walls and white, spongy, bitter tissue and filled with sweetly acid red juice. The extraction process is deeply described by Alper et al. (2005). The pomegranates were washed with water to remove any surface dirt and superficial microbial flora. The fruits were cut into halves, the seeds removed and pressed with a pilot basket press in order to have a 40–45% yield. The extracted juice was sequentially flocculated with 300 mg/L of gelatine A (Sigma–Aldrich Co., Bloom: 90–100) and 300 mg/L of Bentonite (Sigma–Aldrich Co.), at room temperature for 1 h. Clear juice was obtained by filtration through cheese paper. Samples of 50 ml were stored frozen and thawed at room temperature before the experiments.

2.2. High pressure apparatus

The experiments were carried out in a high pressure (HP) pilot plant MINI FOODLAB FPG5620, constructed by Stansted Fluid Power Ltd. (Stansted, UK). Designed for the high pressure treatments at temperatures ranging from −20 to 90 °C, the HP apparatus has an internal volume capacity of 0.5 L, generally reduced to 0.3 L due to a perforated cylindrical gasket used for the samples load and recovery. The maximum operating pressure is 900 MPa and the pressurization fluid is a 70% ethanol–water solution mixed with castor oil used as lubricant. After loading the samples and setting the processing conditions in terms of pressure and temperature levels, pressurization rate, holding time under pressure, as described in the experimental procedure paragraph, the HP apparatus automatically carries out the treatment, allowing the recovery of the treated samples at the end of the decompression step and the lighting of the secure opening lamp. The system is completely adiabatic and causes an average temperature increase of 4 °C/100 MPa.

2.3. Experimental procedures

The pressure and temperature profiles were recorded for each test. The investigated variables were: pressure (400–600 MPa), temperature (25–50 °C) levels and operating time (5, 10 min). Pomegranate juice samples, thawed before the process, were sealed in flexible pouches made from a multilayer polymer/aluminum/polymer film (polyethylene–aluminium–polypropylene). The pouches were introduced into the high pressure reactor and the pressure cycle set to the chosen experimental conditions (pressure, temperature, holding time). At the end of the treatment, the pouches were stored at 4 °C before the complete chemical–physical and microbiological characterizations. The analytical determinations were carried out in triplicate. Analysis of variance (ANOVA) was used to test effects of pressure, temperature and processing times on the microbial survival ratio and on the chemical–physical parameters of the juice samples (fresh and processed juices). Student tests were used as paired comparisons between sample means. Level of significance was set to 0.05. In the following paragraphs the experimental procedures are described in detail.

2.4. Microbiological assay

The number of surviving cells, N, after each test was determined, after a proper dilution of the treated samples in distilled water, by plate count method. The count of microbial colonies, grown on PCA (Plate Count Agar, Oxoid) slants at 32 °C for 72 h, was expressed in cfu/ml (colony forming units per ml of sample). The survival fraction, $S = N/N_0$, and the level of inactivation, $\log(S)$ were evaluated for each test.

2.5. Chemical–physical characterization

In order to determine the effectiveness of the HPP, the main parameters related to the juice quality were estimated for both the untreated juice samples as well as the samples processed under different combinations of the processing parameter. In particular color on *CIELAB* scale (L^*, a^*, b^*), TCD (total color density), PC (Polymeric Color), CDT (Color Due to Tannins), AC (Anthocyanins Color), IB (Browning Index), turbidity, total phenols and aroma were measured in triplicate for each sample.

2.5.1. Color analysis

A tristimulus colorimeter Chroma Meter CR-200b (Konika Minolta Sensing Inc., Osaka (Japan)) was used to measure the color of the pomegranate juices. The color is expressed in a three-dimensional color space, which simulates the perception of color by the human eye. The L^* axis (luminance) expresses the brightness, ranging from total black to total white. The a^* and b^* axes are the two color coordinates, with the a^* axis ranging from green to red and the b^* axis ranging from yellow to blue. For the measure juice samples were disposed in a plastic container, previously used for the instrument calibration on the standard white plate. For each measurement the chromameters measuring head was placed on the free surface of the juice and the light source was activated. The values of the chromatic parameters were read on the digital display of the instrument.

2.5.2. TCD, PC, CDT, AC analysis

A double-beam UV–vis spectrophotometer V670 (Jasco-Europe, Italy) was used to measure the chromatic characteristics of the juice samples, according to the procedure reported by Alper et al. (2005) for the characterization of pasteurized pomegranate juice.

TCD is expressed as the total absorbance values of the brown compounds, which show maximum absorbance at 420 nm and absorbance of the juice that gives its maximum at 533 nm. The pomegranate juices were diluted with distilled water for each test in order to obtain an absorbance below 1.0 (preferentially between 0.4 and 0.6) measured at 533 nm. The TCD is estimated by the Eq. (1), as a function of the absorbance at 420 nm (Abs420), at 533 nm (Abs533) and at 700 nm (Abs700), and of the Dilution Factor (DF).

$$\mathrm{TCD} = [(\mathrm{Abs420} + \mathrm{Abs533}) - 2(\mathrm{Abs700})] * \mathrm{DF} \quad (1)$$

Polymeric tannin pigments are resistant to the bleaching effect of bisulfate. For this test, 6 mL of clear juice was transferred into two tubes. Distilled water (0.4 mL) was added to the first tube (control) and 0.4 mL of a 20% (w/w) solution of $K_2S_2O_5$ was added

to the second tube. The polymeric color of the juices was measured by using juices added with the bisulfate solution, according to Eq. (2).

$$PC = [(Abs420 + Abs533) - 2(Abs700)] * DF \quad (2)$$

The CDT parameter is evaluated as the ratio of polymeric color and total color density values, according to Eq. (3).

$$CDT(\%) = \frac{PC}{TCD} * 100 \quad (3)$$

The contribute of the anthocyanins concentration was measured by a spectrophotometric analysis carried out at the maximum peak of absorbance of the pomegranate juice. In particular, the measure was carried out at 533 nm.

2.5.3. Browning Index

The Browning Index (BI) represents the purity of brown color and is reported as an important parameter in the processes where enzymatic or non-enzymatic browning takes place (Guerrero et al., 1996; Castañón et al., 1999). It is measured according to Eq. (4) and depends on the colorimetric parameters a^*, b^* and L^*:

$$BI = [100(x - 0.31)]/0.172 \quad (4)$$

where x is given by Eq. (5):

$$x = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*) \quad (5)$$

2.5.4. Turbidity

The turbidity of the pomegranate juice samples was measured by means of a standard double-beam UV–vis spectrophotometer V670 (Jasco-Europe, Italy) at a fixed wavelength of 700 nm.

2.5.5. Colorimetric determination of total phenols

The Folin–Ciocalteu assay was used for the determination of total phenol content in the pomegranate juices. This assay is often used to determine the total content of food phenols, especially of red wines. The total phenols were determined colorimetrically. A mixture of distilled water and reagents was used as a blank. The phenols are expressed as gallic acid equivalents. Gallic acid standards at five different concentrations ranging from 100 to 500 mg/L were prepared. By using these standards, the gallic acid calibration curve was obtained, with the total phenols being calculated from this curve.

2.5.6. Aroma

The aroma of the fruit juice was tested by means of an electronic nose. Experiments were carried out with a portable electronic nose, PEN2 (PCA Technologies, Milan (Italy)). PEN2 consisted of a sampling apparatus, a chamber containing a series of sensors, and pattern recognition software (Win Muster v.1.6) for data recording. For each test, 10 ml of the liquid samples were closed in a sealed flask (internal volume: 50 ml) endowed with an inlet and outlet valve. Flask were left for 20 min at ambient temperature to reach a stable vapor pressure of volatile compounds. The flask headspace was fluxed inside the sensor chamber of the instrument by means of the measuring probe connected to the outlet valve place of the flask. Considering the preliminary conditioning of the instrument (flushing time: 400 s), a single measurement was carried out in 10 min. Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) statistical analyses were used to elaborate the experimental data and discriminate the odor of the samples.

3. Results and discussion

3.1. General aspects

In order to test the effect of high pressures on the stabilization as well as nutritional and sensorial properties of red fruit juices, HP cycles were carried out on samples of pomegranate juice under different operating conditions. The performed research activity will create general criteria for the selection of process conditions in terms of the time–pressure–temperature combination able to ensure the preservation of the functional properties of pomegranate juice. This will be achieved through a detailed analysis of the experimental results deriving from microbiological, chemical and sensorial determinations.

3.2. Effect of processing conditions on microbial load

The samples of pomegranate juice, characterized by an initial microbial concentration of approximately 10^4 cfu/ml, were treated by high hydrostatic pressure. Despite the initial high microbial load, probably due to the poor quality of the pomegranate fruits used for the extraction of the juice, the microbiological assays performed on the treated samples show that the microbial load after the HP cycles is always lower than 1 cfu/ml. This result highlights the efficacy of the HP process, whatever pressure and holding time are set for the experiment and confirm the experimental data reported in literature for acidic fruit juices (Di Matteo et al., 1996; Donsì et al., 1998; Linton et al., 1999; Alpas et al., 2000; Donsì et al., 2003). According to the experimental results achieved, the residual microbial load and therefore the microbiological criteria do not give any useful indications on the optimal operating condition. All the combinations of pressure, temperature and holding time should be taken into account in the following experiments. Moreover, the chemical–physical parameters provide sufficient information for the choice of the optimal process conditions.

3.3. Effect of processing conditions on chemo-physical and nutraceutical properties

3.3.1. Effect on pomegranate juice color: CIELAB scale

In Table 1, the values of the color parameters on the CIELAB scale (L^*, a^*, b^*) were listed for raw (Reference) and HP treated samples as a function of the operating pressure and holding time at three different temperatures (25, 45 and 50 °C). At room temperature, the luminosity of the samples is stable and seems not to be affected by the treatment time and pressure level. The processing temperature, instead, is ineffective only at values lower than 45 °C. If the processing temperature is set at 50 °C, the luminosity of the samples is halved in respect to the values considered for the fresh juice, whatever the operative pressure and holding time are. Among the two chromatic parameters, a^* and b^*, the variation of the a^* value is more representative of the impact of the process on the appearance of the fruit juice. The a^* parameter, representing the redness of the sample, can give significant information about the content of the natural pigments, responsible for the typical color of pomegranate juice. At room temperature the pressurization of the juice sample contributes to increasing the a^* values, while the processing time has an irrelevant effect on the intensity of red color of the juice. If a mild heating is coupled with the pressurization effect, the pressurization always causes an increase of the redness of the juice in respect to the reference sample, but the variation of the a^* value does not depend on the pressure level applied. Whatever pressure level is tested, the pressure treatment improves the color of the juice independent of holding time, with the redness of the treated samples being similar for the three pressure levels

Table 1
Values of color parameters on CIELAB scale of pomegranate juice samples as a function of the processing pressure at different temperature levels and treatment times.

Reference	T = 25 °C, t = 5 min				T = 45 °C, t = 5 min				T = 50 °C, t = 5 min			
	L^*	a^*	b^*	ΔE	L^*	a^*	b^*	ΔE	L^*	a^*	b^*	ΔE
	41,10	11,97	2,87	57,47	41,10	11,97	2,87	57,47	41,10	11,97	2,87	57,47
400 MPa	39,83	14,40	4,50	59,60	40,33	15,40	4,63	59,33	27,80	20,22	9,11	47,96
500 MPa	40,23	16,40	5,37	59,77	40,63	16,53	5,23	58,93	25,62	19,21	6,56	48,72
600 MPa	41,60	13,93	4,13	57,93	40,10	15,70	5,07	59,40	28,41	17,51	7,44	49,24
	T = 25 °C, t = 10 min				T = 45 °C, t = 10 min				T = 50 °C, t = 10 min			
	41,10	11,97	2,87	57,47	41,10	11,97	2,87	57,47	41,10	11,97	2,87	57,47
400 MPa	40,53	11,63	2,63	58,33	40,20	15,83	4,80	59,63	28,12	18,76	8,27	51,92
500 MPa	43,67	18,60	7,50	57,53	40,73	16,63	5,37	58,87	27,57	19,59	7,21	47,71
600 MPa	44,50	19,57	8,30	57,17	39,97	15,63	4,83	59,50	27,96	18,80	7,41	46,92

tested. The analysis of the trend of the total color difference, ΔE, conveys the same indications. Despite the constant values observed for the samples treated at a temperature lower than 45 °C in respect to the raw juice, significant variations are observed only at temperature values of 50 °C. Therefore, among the combinations of the processing parameters tested, the appearance and the color perception of the juice are noticeably influenced by the high pressure process at a temperature higher than 45 °C. The color analysis on the CIELAB scale suggests that the processing temperature is the parameter controlling the effect of the HP process on juice appearance. Moreover, the lower the processing temperature, the more negligible the overall color variation of the pomegranate juice is. The trend observed for the a^* parameter also indicates that the content of the free molecules responsible for the redness of the juice may change after a high pressure treatment.

3.3.2. Effect on pomegranate juice color: spectrophotometric analysis

In order to highlight the correlation between juice composition and color variation observed after a high pressure treatment as well as intensify the effect of the process on food quality, the color of the fresh and HP treated juices was determined through a detailed spectrophotometric analysis, as reported by Alper et al. (2005). The total color density (TCD), color density related to tannins (CDT), the color density related to polymeric substances (PC) of the samples were determined under several operating conditions. The experimental values of TCD parameter are shown in Fig. 1 for the raw and HP samples of pomegranate juice. The analysis of the experimental data clearly indicates the contribution of pressure, holding time and temperature on the sample color. At room temperature an increase of the pressure level applied causes a decrease of the value of this parameter dependent on the processing time. While a slight variation is observed for the samples processed at room temperature and for a holding time of 5 min, the TCD values linearly decrease in function of the operating pressure down to 70% of the initial value at the higher pressure level tested. Whereas, different trends are observed for the TCD values estimated for the HP samples treated at 45 and 50 °C. First of all, at both the temperature levels, the TCD values do not depend on the processing time, with the experimental data being comparable and a threshold level of the processing pressure detected above which the trend of the TCD values is reversed. At 45 °C, for instance, the TCD values start to increase with pressure level above 500 MPa, while the pressure threshold value is 400 MPa at an operating temperature of 50 °C. However, the total color density takes into account the contribution of the compounds capable of determining the color perception of pomegranate juice, such as tannins, anthocyanins and polymeric fraction. Among these parameters, the tannins contribute to defining the astringent taste typical of both red wine and pomegranate juice, even if the effect of the high pressure process on this compound is not well understood. The values of the CDT are plotted for raw and processed juices as a function of the processing pressure, time and temperature in Fig. 2. At room temperature, the pressure cycles, i.e., pressure levels and holding time, have no significant effects on CDT, whatever the pressure level and processing time tested are. Similar results are achieved if the operating temperature is increased up to 45 °C. Moreover, the trends of the CDT values are overlapped for the samples processed at room temperature and 45 °C. A different behavior is shown by the samples treated at 50 °C. In this case, pressurization causes an increase of the CDT value in respect to the value estimated for the raw sample. The observed increase of this parameter is higher for the samples treated at 400 MPa than the samples treated at 500 MPa and 600 MPa, which have a similar CDT value. Independent of the pressure level applied, the trends of the CDT parameters are similar for both the processing times tested, confirming that the observed increase of this parameter is mainly

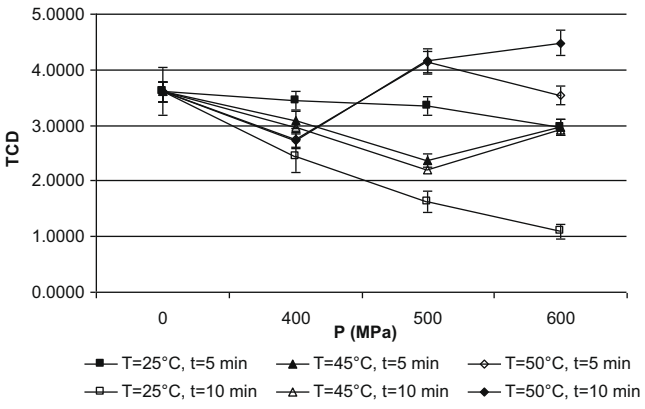

Fig. 1. Total color density (TCD) values of pomegranate juice samples as a function of the processing pressure at different temperature levels and treatment times.

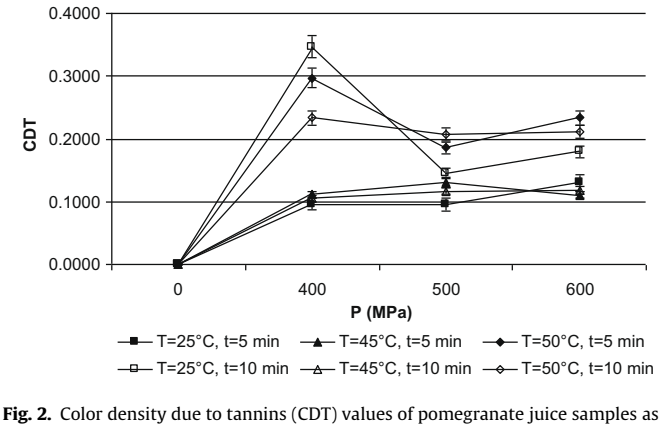

Fig. 2. Color density due to tannins (CDT) values of pomegranate juice samples as a function of the processing pressure at different temperature levels and treatment times.

correlated to the increase of the processing temperature. According to this observation, the increase of the TCD parameter is partly related to the content of the free tannins and the operating temperature should be controlled in order to reduce the astringency of the juice after the high pressure processing, while pressure and processing time seem to be ineffective on the this sensorial characteristic.

3.3.3. Browing Index and turbidity

In Fig. 3, the behavior of the Browning Index, BI, is plotted as a function of the pressure level applied, upon varying the processing conditions (holding time, operative pressure). BI represents the purity of the brown color, as observed in several papers (Guerrero et al., 1996; Castañón et al., 1999) and depends on two main parameters: the operative temperature and the processing time. If the processing time is set at 5 min, the high pressure process does not affect the values of the color parameters at room temperature, as the BI shows a constant trend. If the operative temperature increases at a parity of processing time, a significant variation of the brown color can be detected, only if the pressure level applied exceeds 500 MPa and the operative temperature is higher than 45 °C. At longer processing times, the trend of the BI parameter changes as a function of the operative temperature. At room temperature the measured values decrease with the pressure level, while at an operative temperature of 45 °C a significant variation of the color parameters cannot be detected. Finally, at a temperature of 50 °C, the BI value increases with the pressure level applied. These results clearly demonstrate that the color stability of pomegranate juice depends on the processing conditions. However, the increase of the BI values at the higher pressure and temperature levels confirms the trend observed for the TCD parameter. Therefore, spectrophotometric and colorimetric determination both indicate that the color of the juice moves towards the higher intensity of red color, while the loss of the luminosity of the juice demonstrates that the color of the juice becomes darker. The turbidity of the samples, even if it is negligible in the unpasteurized pomegranate juice due to the effective clarification performed before the HP treatment, is drastically reduced after the process, whatever the processing conditions tested are. High pressure contributes to mechanically destroying the complexes responsible for the turbidity of the samples and not-detectable by the UV–vis spectrophotometer. The values of the turbidity of pomegranate juice samples processed under different conditions are plotted in Fig. 4. Therefore, the HP treatment contributes to stabilizing the clearness of the juice and avoids the separation of solid particles or complexes generally observed for fruit juices during storage.

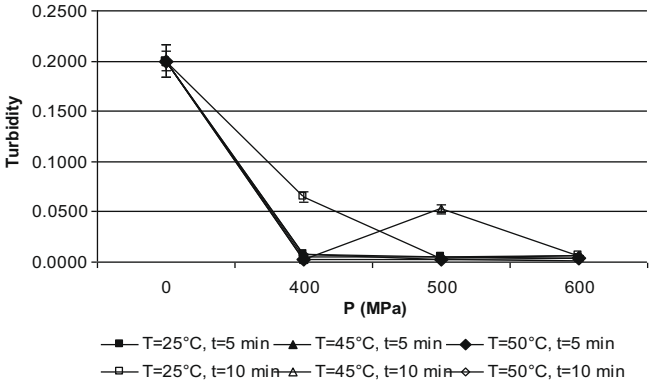

Fig. 4. Turbidity values of pomegranate juice samples as a function of the processing pressure at different temperature levels and treatment times.

3.3.4. Effect on the nutraceutical compounds: anthocyanins and polyphenols

The concentration of anthocyanins and the content of the total phenols were determined for the HPP juices and compared with the values estimated for the fresh juice. The results are shown in Fig. 5 for the total amount of anthocyanins (AC) of high pressure treated samples at three different levels of temperature (25, 45, 50 °C) and two treatment times (5, 10 min). In Fig. 5, the value estimated for the fresh juice corresponds to the zero pressure level. The experimental results indicate that the content of anthocyanins is influenced mainly by pressure and temperature level. At room temperature, the concentration of these molecules decreases with the intensity of the treatment in terms of pressure level and processing time. Therefore, the higher pressure levels or longer processing times cause both a decrease of the anthocyanins content. If the samples undergo a treatment at a temperature higher than room value, the experimental data follow a different trend. First of all, the treatment time is not a relevant parameter, due to the concentration of the anthocyanins being similar for both the processing times tested. The application of the high pressure causes a slight decrease of the AC content, which results being independent of the pressure level tested at mild temperature (45 °C). If the operating temperature exceeds 45 °C and the pressure level is higher than 400 MPa, the observed trend is reversed. The AC content, in fact, is similar or higher than the value estimated for the fresh juice. This result indicates that in this particular range of processing conditions the high pressure treatment mainly modifies the mechanism of anthocyanins degradation by affecting the molecules involved in the kinetics of reaction, such as enzymes. Moreover, the observed trend of the AC content is similar to the one

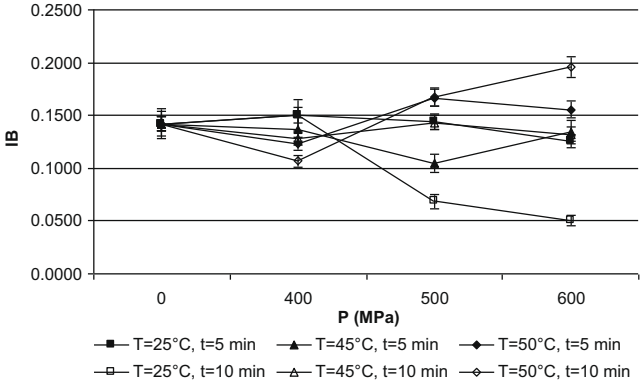

Fig. 3. Browning Index (BI) values of pomegranate juice samples as a function of the processing pressure at different temperature levels and treatment times.

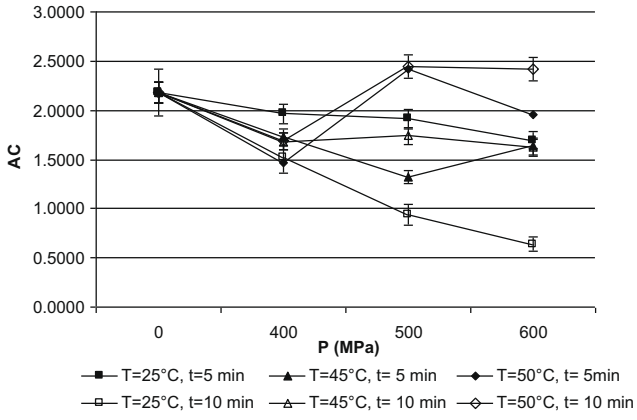

Fig. 5. Total anthocyanins (AC) values of pomegranate juice samples as a function of the processing pressure at different temperature levels and treatment times.

Table 2
Total Polyphenol content (g/L of gallic acid) values of pomegranate juice samples as a function of the processing pressure at different temperature levels and treatment times.

Reference	T = 25 °C, t = 5 min Total polyphenol content(g/L of gallic acid)	T = 45 °C, t = 5 min Total polyphenol content(g/L of gallic acid)	T = 50 °C, t = 5 min Total polyphenol content(g/L of gallic acid)
	1,36	1,36	1,36
400 MPa	1,04	1,15	1,48
500 MPa	1,14	1,18	1,30
600 MPa	1,19	1,02	1,15
	T = 25 °C, t = 10 min	T = 45 °C, t = 10 min	T = 50 °C, t = 10 min
	1,36	1,36	1,36
400 MPa	1,27	0,95	1,92
500 MPa	0,89	1,13	1,38
600 MPa	0,79	1,08	1,32

already described for the TCD parameter. Therefore, the color density of the juice is correlated to the concentration of free anthocyanins, which can represent the key factor for the standardization of the processing conditions in high pressure treatments. Several authors have reported that anthocyanins are stable to HP treatment at moderate temperatures (Patras et al., 2009b). In contrast, some authors have also reported increased extractability of colored pigments in food components at extreme pressures (Sánchez-Moreno et al., 2005; Patras et al., 2009a). However the observed stability of these molecules could be transient. The pressure and temperature levels tested during the experiments do not allow an irreversible inactivation of the enzymes involved in the degradation of the natural pigments. Therefore, the residual activity of the enzymes along with a small concentration of dissolved oxygen could cause the degradation of the anthocyanins during the storage of the processed juice, as widely supported by studies reported in current scientific literature (Zabetakis et al., 2000; Suthanthangjai et al., 2005). These results confirm the experiments carried out at room temperature and allow the data obtained at higher temperature to be explained. In other words, the results showed that enzyme activity decreased when the temperature increased, as already been observed by several authors (Fang et al., 2008).

The content of the total polyphenols was also determined for fresh juice and high pressure processed samples, with the results achieved at different pressures and temperatures as well as processing times being listed in Table 2. The behavior of this parameter is similar to the one observed for the anthocyanins content, due to the treatment being time relevant at room temperature and the pressure level decreasing the value of the total polyphenols. A temperature level higher than 45 °C modifies the trend shown by the measured parameter. In particular, the application of a pressure level of 400 MPa increases the total polyphenols content, while at higher pressure levels the measured polyphenols content of the processed samples is comparable to the value estimated for the fresh juice. While the trend observed at room temperature and at 45 °C can be explained as in the case of anthocyanins, by taking into account the enzymatic residual activity, with particular attention being attributed to the results obtained at a temperature

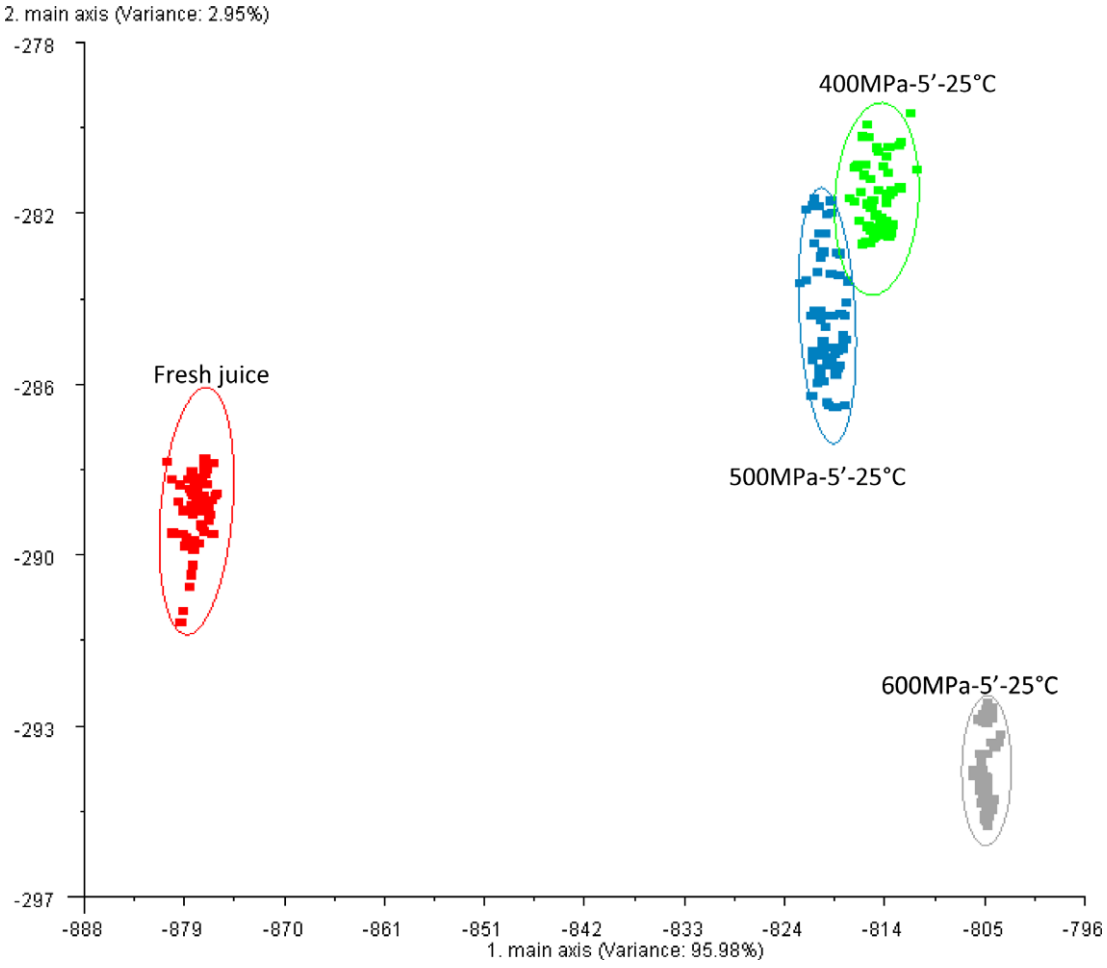

Fig. 6. Aroma pattern of pomegranate fresh juice and HP samples processed at different pressure level at ambient temperature (25 °C) and fixed treatment time (5 min).

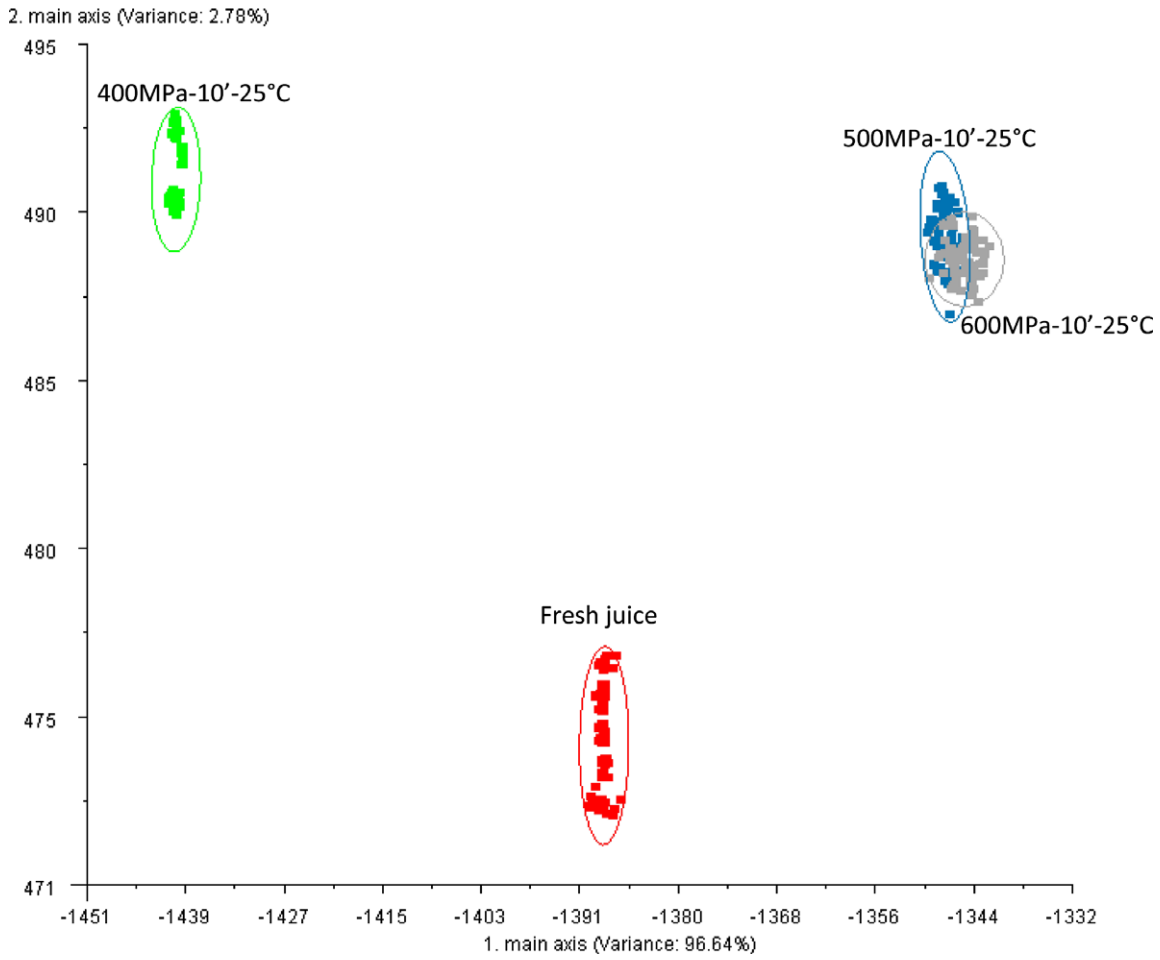

Fig. 7. Aroma pattern of pomegranate fresh juice and HP samples processed at different pressure level at ambient temperature (25 °C) and fixed treatment time (10 min).

of 50 °C. The increase of the polyphenols concentration has already observed for red fruit derivatives or fresh fruits treated by high hydrostatic pressure in recent studies (Corramles et al., 2008; Patras et al., 2009a; Terefe et al., 2009). Therefore, the content of the nutraceutical compounds can be used for the discrimination and choice of the optimal processing conditions to be used for the treatment of the pomegranate juice.

3.3.5. Effect on the pomegranate juice aroma

Finally, the effect of the process on the aroma of the juice samples is described in detail in Figs. 6–8, in which the aroma of the fresh and high pressure processed samples are analyzed according to the chemometric approach. Several mathematical methods could be applied to the multi-component analysis of the odors. The LDA approach was used to analyze the aroma pattern of the tested samples. The Electronic Nose (EN) pattern of the HP processed juices was set up under three different conditions. Fig. 6 compares the EN pattern of the fresh juice and HP samples processed at several pressure levels and a fixed treatment time (5 min). This pattern allows approximately 99% of the aroma variance to be represented. In particular, the first main axis corresponds to an overall variance of approximately 96%. Therefore, the variations observed along this axis represent the differences among the aroma of the sample better. The second main axis is associated to an overall variance of approximately 3%. Therefore, the variations along this axis may be considered negligible. The LDA analysis, in this case, allows the aroma of the fresh and processed samples, which take up separate zone of the plot, to be discriminated. Moreover, the set of data corresponding to the different pressure levels show the same value on the first main axis, while differ on the value of the second main axis. The data related to the samples processed at 400 MPa and 500 MPa are partially overlapped, thus demonstrating that the aroma of the samples are similar. Even if the samples processed at 600 MPa show a different aroma profile, taking into account the variance of the second main axis, it can be concluded that the aroma of the HP juices can be distinguished from that of the fresh juice, while the pressure level has no significant effect on the aroma profile. Similar observations derive from the analysis of the EN pattern plotted in Fig. 6 for the fresh juice and HP juice samples treated for a longer processing time (10 min). In this specific case, higher processing times are effective on the aroma of the samples processed at the lower pressure level tested, while the aroma profile of the samples processed at 500 MPa and 600 MPa are completely overlapped. Finally, Fig. 8 reports the EN profile of the fresh juice and HP samples processed at a fixed pressure level (400 MPa) and processing time (5 min) but at different temperature levels. The EN pattern allows the aroma of the processed and fresh juices to be discriminated. It also demonstrates that the operating temperature is a relevant parameter. The samples processed at different temperatures have a different aroma profile. In conclusion, the aroma of pomegranate juice is mainly influenced by the operating temperature and processing time. The application of high pressure has no significant effect on the aroma at room temperature and a short processing time. The increase of the operating temperature or processing time

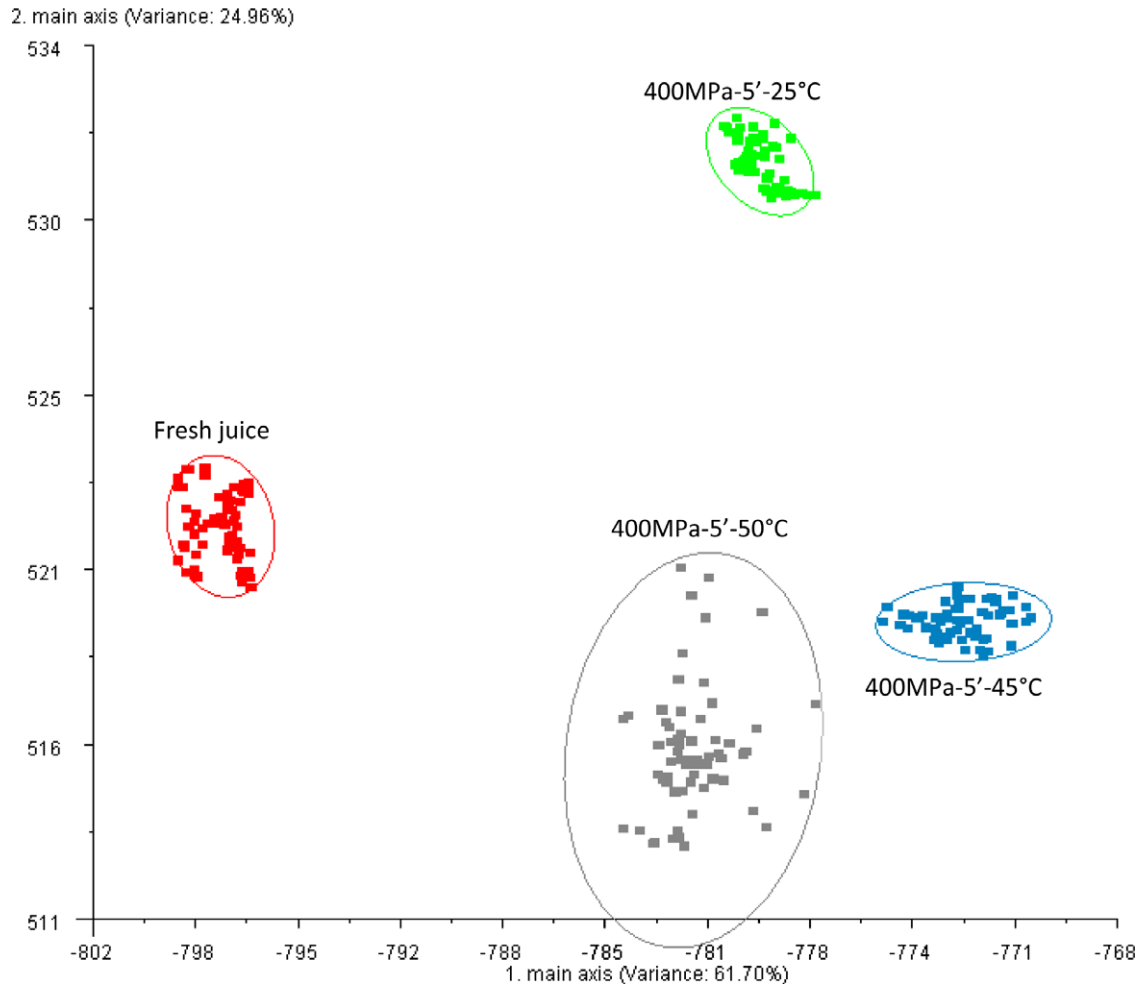

Fig. 8. Aroma pattern of pomegranate fresh juice and HP samples processed at fixed pressure level (400 MPa) and processing time (5 min) and different temperature levels.

causes an evident variation of the aroma with respect to the fresh juice as reported in current literature (Lambert et al., 1999).

4. Conclusions

The application of high pressures results to be particularly interesting on the pomegranate juice, characterized by a high added value, due to the high content of nutraceutical components (anthocyanins, polyphenols, tannins). The experiments, by analyzing the effect of the main processing variables (pressure, temperature and time) on both the microbiological stability as well as the properties responsible for the sensorial and nutraceutical quality of the investigated products, demonstrate that a pressure level greater than 400 MPa ensures the complete decontamination of pomegranate juice. In agreement with the experimental data, the main process variable was found to be operating temperature. In particular, since at the maximum temperature used in the tests of 50 °C, the color indices L^*, a^* and b^* differ significantly from the values presented by the reference samples as well as significant variation of the values of BI and CDT, the content of polyphenols and the aroma, it is arguable that the stabilization of pomegranate juice cannot be carried out at 50 °C. The differences between the experimental results performed on samples processed at 45 and 25 °C are significantly minimal. Therefore, the choice lies in room temperature, thus saving the costs relating to the heating of the high pressure reactor and reducing the occurrence of heat-induced modification of juice quality. Since the increase of the processing time or pressure level causes the reduction of nutraceutical compounds after the high pressure treatment at room temperature, this suggests minimizing the value of these parameters. Therefore, in the specific case of the pomegranate, very mild processing conditions can be applied in order to ensure the safety of the product as well as preserve the natural quality of the product. Further experiments are required in order to verify the stability of pomegranate juice during storage under refrigerated conditions as well as to define the shelf-life of the product processed under optimized treatment conditions.

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