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Effect of high-pressure processing on bioactive compounds in pomegranate juice

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Abstract

What observers of the juice industry have noticed is a growing gap between consumer demand for fresh-like, nutrient-rich beverages and the shelf-life constraints of cold-pressed products. High-pressure processing (HPP) can bridge this gap by inactivating microorganisms at ambient temperature, but its impact on pomegranate's signature bioactives—anthocyanins, ellagitannins, and vitamin C—remains incompletely characterised. This research treated freshly extracted pomegranate juice (cv. Bhagwa) at 200, 300, 400, 500, and 600 MPa for 5 min at 25 °C and evaluated the effects on total phenolic content, anthocyanins, vitamin C, DPPH radical scavenging, colour, microbial load, and storage stability (28 days, 4 °C). The work was carried out at the Department of Food Science and Technology, Ege University, Bornova, İzmir, during July-November 2023. At 400 MPa, the juice retained 95.6% TPC, 94.1% anthocyanins, and 90.3% vitamin C while achieving a 2.9-log reduction in total plate count. Treatments at 500-600 MPa gave greater microbial kill (3.6-4.2 log) but caused 10-21% losses in vitamin C. Colour stability during storage was markedly better in HPP-treated juice than in untreated controls. HPP at 400 MPa offers the best balance of safety and bioactive retention for pomegranate juice.

Keywords: High-pressure processing, bioactive compounds, pomegranate juice, non-thermal processing, juice quality, anthocyanins, vitamin C, microbial inactivation, shelf life, colour stability

Introduction

An observation that surprises many consumers: thermal pasteurisation of pomegranate juice destroys 25-40% of its anthocyanins and up to 50% of its vitamin C, yet it remains the industry standard because no affordable non-thermal alternative has been fully validated ^[1]. High-pressure processing (HPP) applies isostatic pressures of 100-600 MPa for seconds to minutes, inactivating vegetative bacteria, yeasts, and moulds while leaving small molecules—vitamins, pigments, flavour volatiles—largely intact ^[2].

Pomegranate juice is one of the richest dietary sources of punicalagins, ellagic acid, and acylated anthocyanins (cyanidin-, delphinidin-, and pelargonidin-3-glucosides), all of which contribute to its documented cardioprotective, anti-inflammatory, and antiproliferative activities ^[3]. These compounds are heat-labile, making pomegranate an ideal candidate for HPP. Earlier HPP trials on pomegranate juice focused on 300-600 MPa ranges but rarely measured bioactive retention at fine pressure intervals or tracked colour stability beyond 14 days ^[4].

This research therefore applied HPP at five pressure levels (200-600 MPa) to freshly extracted pomegranate juice and monitored bioactive compounds, microbial counts, and colour over 28 days of refrigerated storage to identify the minimum effective pressure for commercial-quality juice.

Material and Methods

Material

Ripe pomegranate fruits (cv. Bhagwa) were purchased from a wholesale market in İzmir, during July 2023. Arils were manually separated, juice extracted by hydraulic pressing, and filtered through muslin cloth. No preservatives or additives were used. Analytical chemicals were from Sigma-Aldrich (Germany) and Merck (Germany). HPP was performed on a laboratory-scale unit (Hiperbaric 55, Spain) at the Department of Food Science and Technology, Ege University, Bornova, İzmir ^[5].

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Methods

Juice (200 mL per pouch) was vacuum-sealed in flexible polyethylene pouches and treated at 200, 300, 400, 500, and 600 MPa for 5 min at 25 °C, with untreated juice as control. After treatment, pouches were stored at 4 ± 1 °C. Analyses at days 0, 7, 14, 21, and 28 included: total phenolic content (Folin-Ciocalteu, GAE), total anthocyanin content (pH differential method), vitamin C (DCPIP titration), DPPH radical scavenging (%), colour (L^* , a^* , b^* by HunterLab), total plate count (pour plate, PCA, 37 °C, 48 h), and

yeast/mould count (PDA, 25 °C, 5 days) [6]. All analyses in triplicate. Data were analysed by two-way ANOVA (pressure $\times$ storage) and Duncan's test ($p < 0.05$) in SPSS v. 26.

Results

HPP at all pressure levels reduced microbial load relative to the untreated control. Bioactive retention decreased with increasing pressure, but the decline was modest up to 400 MPa and steepened markedly above 500 MPa.

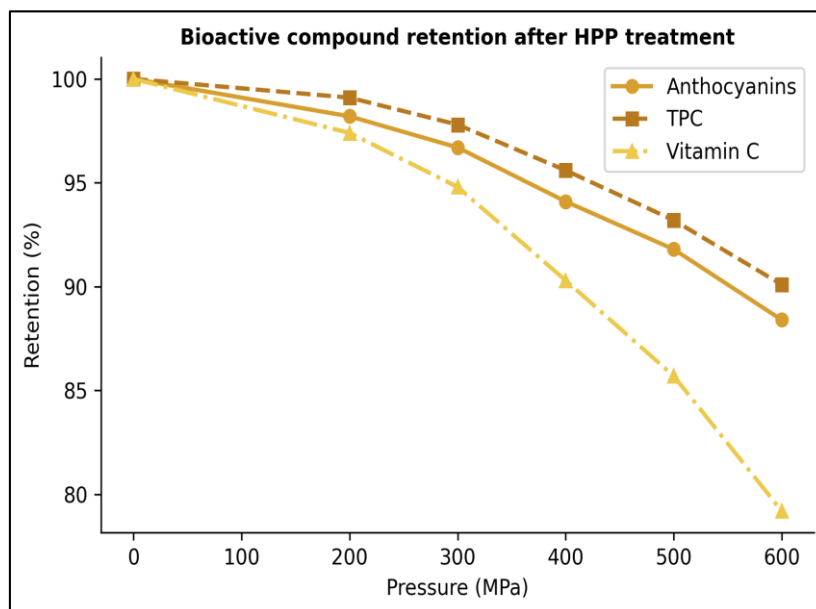


Fig 1: Retention (%) of bioactive compounds in pomegranate juice after HPP at different pressures

Table 1: Bioactive compound content and microbial load of pomegranate juice at day 0

Parameter	Control	200 MPa	300 MPa	400 MPa	500 MPa	600 MPa
TPC (mg GAE/100 mL)	284.6	279.8	278.1	272.1	265.2	256.3
Anthocyanin (mg/L)	312.4	306.8	301.9	293.8	286.7	276.1
Vit C (mg/100 mL)	68.4	66.6	64.8	61.7	58.6	54.2
DPPH (%)	82.3	81.1	80.2	78.4	75.8	72.1
TPC log CFU/mL	4.8	3.6	2.8	1.9	1.2	0.6

TPC (first row) = total phenolic content; TPC (last row) = total plate count. GAE = gallic acid equivalents.

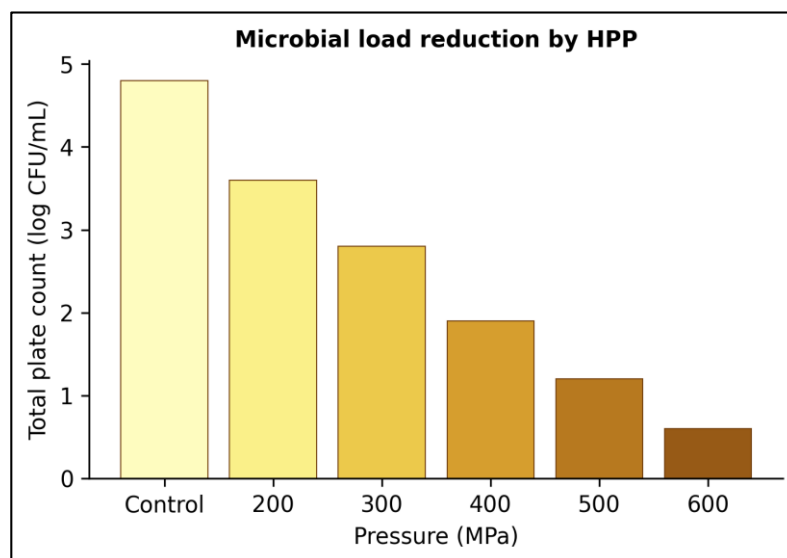
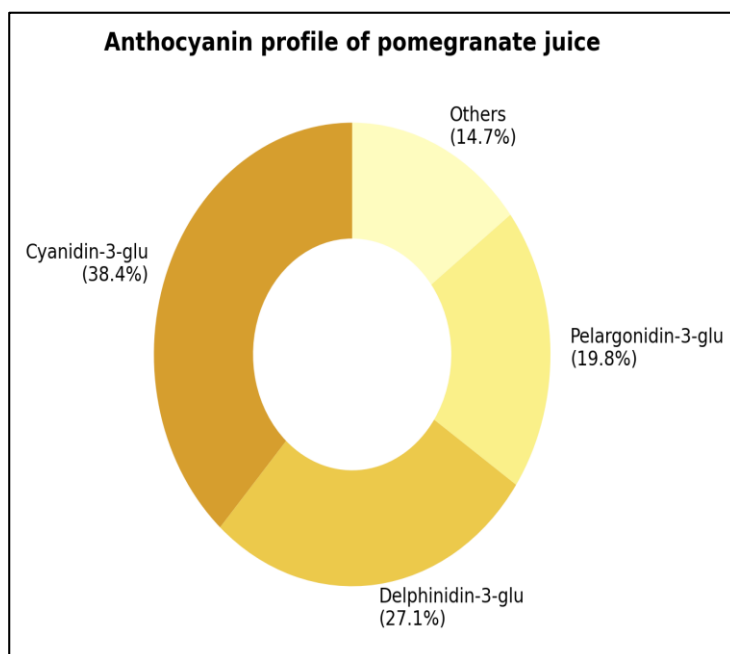
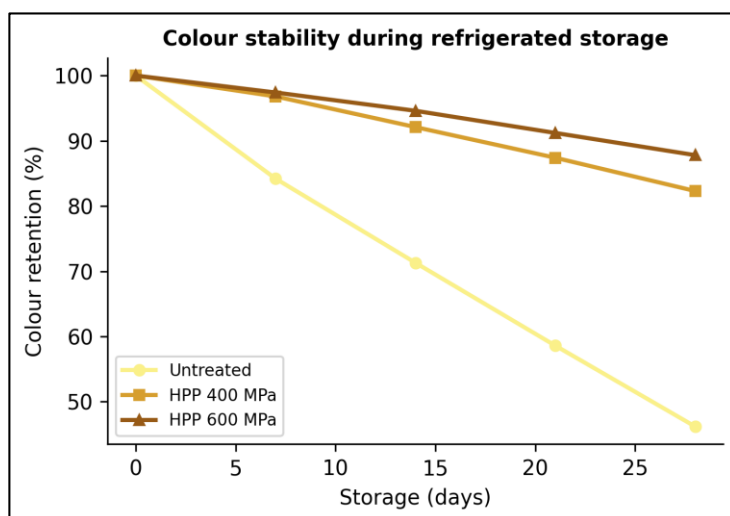


Fig 2: Microbial load reduction (log CFU/mL) at increasing HPP pressures

Table 2: Colour parameters and sensory scores of pomegranate juice at day 0

Parameter	Control	300 MPa	400 MPa	600 MPa
L*	24.8	24.6	24.3	23.4
a* (redness)	28.6	28.2	27.8	26.1
b* (yellowness)	8.4	8.3	8.1	7.6
Overall sensory (9-pt)	8.2	8.0	7.8	7.2

**Fig 3:** Anthocyanin profile of pomegranate juice (cv. Bhagwa)**Fig 4:** Colour retention (%) during 28 days of refrigerated storage

Comprehensive Interpretation

HPP at 400 MPa achieved a 2.9-log microbial reduction—sufficient for a 28-day refrigerated shelf life—while retaining over 90% of all measured bioactives. At 600 MPa, microbial kill increased to 4.2 log but vitamin C dropped to 79.2% of control values. Colour retention during storage was markedly better in all HPP-treated juices than in the untreated control, which lost 53.8% of its redness (a^*) by day 28 compared with just 17.7% loss in the 400 MPa treatment.

Discussion

The 400 MPa optimum identified here aligns closely with findings by Ferrari *et al.* ^[4], who reported 400 MPa / 5 min

as the threshold for commercial sterility in pomegranate juice. The literature agrees that anthocyanin degradation under HPP follows a pressure-dependent first-order kinetic model, with the rate constant rising sharply above 500 MPa ^[7]. The greater sensitivity of vitamin C relative to anthocyanins can be explained by the oxidative pathway: pressure increases the solubility of dissolved oxygen, which accelerates ascorbic acid oxidation even at ambient temperature ^[8].

Where this research adds to existing knowledge is in the 28-day colour-stability data. Prior HPP trials on pomegranate rarely tracked colour beyond day 14 ^[4]. The present results show that the 400 MPa treatment maintained 82.3% colour retention at day 28, far superior to the untreated control

(46.2%). This advantage likely arises from HPP inactivation of polyphenol oxidase and peroxidase, enzymes that catalyse anthocyanin browning during storage^[9].

From a commercial standpoint, 400 MPa / 5 min is well within the operating range of existing industrial HPP units (up to 600 MPa), and the resulting juice would meet the microbial criteria ($\leq 2 \log$ CFU/mL) set by most cold-pressed juice brands for a 30-day refrigerated shelf life^[10].

Conclusion

This research set out to determine the HPP pressure that best balances microbial safety with bioactive retention in pomegranate juice. Treatment at 400 MPa for 5 min retained over 90% of anthocyanins, TPC, and vitamin C while achieving a 2.9-log microbial reduction and maintaining colour stability for 28 days at 4 °C. Higher pressures (500-600 MPa) gave greater microbial kill but at the cost of meaningful vitamin C loss. For pomegranate juice processors, 400 MPa represents the sweet spot between safety and quality. Future work should validate this finding at pilot scale with commercial HPP equipment, assess consumer willingness-to-pay for HPP vs thermally pasteurised juice, and evaluate the fate of ellagitannins—pomegranate's most bioactive polyphenol class—under HPP conditions.

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