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# Valorization of Pomegranate Peels as a Healthy Ingredient to Preserve Orange Juice

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ABSTRACT

This study evaluated the possibility to use pomegranate peel: an agro-industrial bay product as a conservative agent for fruit juice. Extraction of active compounds of. *Punica granatum* L. peels was made by maceration with ethanol and the evaluation of antioxidant and antimicrobial activity were conducted using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and disk diffusion method in agar medium, while self-life tests of orange juice monitoring the pH, total soluble solids, titratable acidity, cloud value, browning index and total mesophilic germs were conducted during 18 days. The results highlighted that pomegranate peel extract (PPE) showed a higher phenol and flavonoid content and strong antioxidant activity with an inhibitory concentration ($IC_{50}$) of 6.22 µg / mL, as well as the antimicrobial activity indicated a higher inhibitory effect. Furthermore, shelf life tests showed a significant effect on browning index, titratable acidity, and lowering of microbial growth during storage compared to the controls, while pH, total soluble solids, and cloud value are not affected. These results allow us to consider pomegranate peel extract as a potential conservator for healthy and sustainable food.

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## Introduction

Citrus juice, essentially orange juice, is the predominant beverage around the world (Cortes et al., 2008). Fresh juice deteriorates rapidly after extraction, due to endogenous enzymes and microbial growth from fruit contamination during the production chain **(**Maherani et al., 2018; Mahale et al., 2008). Although chemical reagents such as citric acid and sodium benzoate remain the most widely used preservatives, there is growing interest in the search for biological alternatives that do not have adverse effects on human health and which contribute to improving the organoleptic and nutritional properties of fresh juices (Hassoun et al., 2020).

Due to these food security concerns, special attention has been paid to the development and effective use of naturally occurring, non-toxic antioxidants (Nahas, 2012; Sevindik et al., 2017; Mohammed et al., 2023). In addition, natural antioxidants such as polyphenols, tocopherols, and essential oils have been explored largely from fruit and vegetable wastes, which are the most abundant materials among food products and which account for around 10 to 35 % of the gross mass (Rao and Rathod, 2019)**.** In addition to their beneficial effects through organic food, the recovery of this waste constitutes cinquefoil rents for sustainable development (Mohammed et al., 2022; Uysal et al., 2023).

The pomegranate or "*Punica granatum*" is part of the medicinal and food species, the fruit is composed of four essential parts, namely: the epicarp and the mesocarp commonly called the peel which is not consumable, while the endocarp consists of bags of juice and seeds (called Balausta) are the consumable part of the fruit (Melgarejo et al., 2020). The zest of pomegranates makes up about 50% of the total fruit weight and is an important source of minerals, especially potassium, calcium, phosphorus, magnesium, and sodium, complex polysaccharides, and high levels of a diverse range of bioactive compounds (Singh et al., 2018). It is considered the plant's richest bioactive compounds such as phenolic acids, tannins,

flavanols, and anthocyanins (Li et al., 2006; Masci et al., 2016). This composition has given it several properties both in the medical field and in the food industry (Kandylis et al., 2020)

The application of pomegranates and these derivatives in the agro-food sector has been the subject of research work, which has attempted to evaluate their antioxidant and antimicrobial activities (Akbaş et Kılmanoğlu, 2022 ; Panza et al., 2021; Mastrodi Salgado et al., 2012; Kanatt et al., 2010). Indeed, Konsoula (2016); El-Hadary and Taha (2019) report that the extract of pomegranate peels provides better oxidative stability of edible oils compared to BHT (butyl hydroxytoluene) and TBHQ (tert-butyl hydroquinone), thus samples pomegranate peel extracts in containers retain their antioxidant capacities well for a long time, under accelerated oxidation conditions, indicating that these extracts contain antioxidants offering better protection. The work of Moghadam et al. (2020) has shown that the incorporation of different doses of pomegranate peel extract (PPE) with bean protein results in a biofunctional edible film for the packaging of food products, with good flexibility thus allowing for an increase in the thickness and vapor permeability and lower humidity of packaged foods. The antimicrobial and antifungal activity of pomegranate peel has also been the subject of much research (Panza et al., 2021; Tehranifar et al., 2011; Ismail et al., 2016; Ali et al., 2019) which are effective on a broad spectrum of food borne pathogens such as *Escherichia coli*, *Fusarium sambucinum*, *Penicillium italicum*, and *Bacillus subtilis*. Indeed, Dahham et al. (2010) showed that the antimicrobial activity of pomegranate peel is better compared to that of seeds and whole juice.

The research work reported in the scientific literature mainly in the field of beverages have focused only on the antioxidant and antimicrobial potential of pomegranate peels and seeds (Trigo et al., 2020; Viuda-Martos et al., 2013; Mastrodi Salgado et al., 2012) without any comparison with preservatives used on an industrial scale or monitoring of the physicochemical modifications likely to be caused.

In this context, the present work aims to show the potential of the extract of pomegranate peel as a substitute for chemical preservatives used to extend the shelf life of fruit juices, mainly fresh orange juice, by monitoring the physicochemical and microbiological parameters associated to the conservation.

## Material and Methods

### *Preparation and Extraction of Bioactive Compounds*

The fruits subject to the present experiment were purchased at the local market (Bordj-Bou-Arréridj, Algeria). Oranges and pomegranates which did not have any wounds or external deterioration were washed with tap water to remove any residue. Pomegranate peels were manually separated from the rest of the fruit, then dried in the sun and crushed by an artisanal coffee grinder to obtain a powder of reduced particle size. A constant amount of powder was used to extract the natural antioxidants with a 1: 1 (v / v) ethanol-distilled water mixture for 24 to 48 hours, according to the method optimized by Venkataramanamma et al. (2016). The crude extract was filtered through Wattman paper 45 µm in diameter, and then evaporated to dryness using a rotary evaporator, until the solvent was completely removed.

### *Dosage of Phenolic Compounds*

Total polyphenol determination was carried out according to the method of Folin-Ciocalteu (FC) reported by Oleivera et al. (2014) slightly modified. 0.4 ml of PPE was mixed with 0.4 mL of the FC reagent and 1.2 mL of 2% (m/v) $Na_2CO_3$. The mixture was stirred and incubated in the dark and at room temperature for two hours and the absorbance was measured at 760 nm by a UV-visible spectrophotometer (Perkin Elmer). The results are expressed in mg gallic acid equivalent (GAE) / gram of extract by referring to the calibration curve for gallic acid (1 mg / mL; $R^2 = 0.978$). The total flavonoid content was determined according to the aluminum chloride colorimetric method described by Cedola et al. (2017). 1 mL of 2% methanolic aluminum chloride solution was added to 1 mL of PPE, the solution was incubated for 15 min at room temperature, and then the absorbances were read at 430 nm on a spectrophotometer. Flavonoids content was calculated by referring to the calibration curve obtained using quercetin as a standard (1 mg / mL; $R^2 = 0.978$), and the results are expressed in mg of quercetin equivalent (QE) / g dry extract.

### *In Vitro Bioconservation Test*

*Antioxidant activity*

Antioxidant activity of PPE was performed using DPPH (2,2-diphenyl 1-picrylhydrazyl) as a substrate. This method is based on measuring the capacity of antioxidants to trap the DPPH radical. The effect of PPE extract on DPPH was measured by the procedure described by Brand-Williams et al. (1995). A methanolic solution of 0.1 mM of DPPH (2 mL) is mixed with 1 mL of the diluted extract, and then the mixture is vigorously vortexed. After 30 minutes incubation in the dark, the absorbances were measured at 517 nm and the percentage of DPPH radical scavenging was calculated according to the following equation:

$$\% \text{ Antioxidant Activity} = [(A_1 - A_2) / A_1] \times 100$$

$A_1$: absorbance of the control (DPPH solution without extract).

$A_2$: absorbance of the extract.

The $IC_{50}$ value was determined using linear regression of the curve relating percent inhibition versus PPE concentration (µg / mL) (Martinez-Morales et al., 2020).

*Antimicrobial activity*

The antimicrobial activity of PPE was determined by measuring the inhibitory effect according to the diffusion method on agar medium or the aromatogram technique, which is based on measuring the inhibition zone around a source of antimicrobial agent deposited on the surface of a culture medium. Two pathogenic bacterial strains (*Escherichia coli*, *Bacillus subtilis*), and two fungal strains (*Saccharomyces cerevisiae*, *Accromyces sp*) isolated from orange juice placed under weathering conditions were used. In a Petri dish, from a precise point, with the creation of a concentration gradient after a certain time of contact between the product and the microorganism, 6 mm diameter discs cut from Wattamn N ° 3 paper, sterile are deposited on the gel. A volume of 20 µL of the extract were

deposited on the disks, with a range of decreasing concentrations of 300, 150, 75, 37.5, 18.75, and 9.37 µg / mL. Measurement of the zone of inhibition will occur after incubation at 37° C for 24 hours (Kanoun et al., 2014).

***In Vivo Biopreservation Test***

*Extraction and preparation of orange juice*

Orange juice was chosen as a food for biopreservation trials because it is relatively perishable and is subject to browning reactions, as well as microbial spoilage. Oranges (*Citrus sinensis* L.) freshly harvested in January 2022 were purchased at a local fruit market (Bordj Bou Arreridj, Algeria), whose maturity was characterized by skin color. After rigorous cleaning with distilled water, the oranges were pressed using a domestic fruit extractor (Condor, Algeria), then the ethanolic extract of the pomegranate peel was added to the juice thus obtained at concentrations of D1=0.01, D2=0.02, D3=0.03, and D4=0.04 mg extract/mL of juice. More of these samples, a negative control without PPE and a positive control with citric acid and sodium benzoate at a concentration of 0.003 and 0,001 mg/mL of juice, respectively as preservatives (CODEX STAN, 1995) were followed for 18 days (T ° C ~ 25), by measuring the physicochemical (pH, TSS, titratable acidity, browning index, and cloud value) and microbiological parameters (Enumeration of aerobic mesophilic germs).

***Physicochemical Analysis***

*Total soluble solids and pH*

The pH of the treated and untreated orange juice samples was measured using a digital pH meter (Model 420A, Orion benchtop pH meter, Allometrics Inc.) and the TSS was expressed in ° Brix after determination of refractive index using a refractometer (Abbe 60, Bellingham + Stanley Ltd.).

*Titratable acidity*

Acidity was determined by titration of the juice samples (20 mL of juice + 80 mL of distilled water) with 0.1 N NaOH solution and phenolphthalein as a color indicator. The volume of NaOH was converted to grams of citric acid per 100 mL of juice and the titratable acidity (TA) was calculated using the following formula (Aghajanzadeh et al., 2016):

$$TA = (V \times 0.1N\ NaOH \times 0.067 \times 100) / m$$

Where V is the titer volume of NaOH and m is the mass of orange juice (g).

*Cloud value*

The cloud value of orange juice is related to the suspension of particles made up of proteins, pectins, lipids, hemicellulose, cellulose, and other minor compounds (Tiwari et al., 2008). 5 mL of orange juice was centrifuged (Sigma 2-16P) at 756 g for 10 min at room temperature (20.0 ± 0.5 ° C). The Cloud value was measured as the absorbance of the obtained supernatant at 660 nm using a Unicam UV-Vis spectrophotometer with distilled water as a blank.

***Browning index***

The Browning index was determined according to the method described by Martins et al. (2021). The orange juice was centrifuged at 824x g for 20 min (at 18 ° C), and the resulting supernatant was diluted in ethanol (1:1 v/v), then centrifuged again. The Browning index corresponds to the absorbance of the resulting supernatant at a wavelength of 420 nm (Unicam UV-visible spectrophotometer).

***Microbiological Analysis***

Total mesophilic aerobic organisms were enumerated by surface culture on nutrient agar. For each sample, up to six dilutions ($10^{-1}$, $10^{-2}$, $10^{-3}$,…, $10^{-6}$) were performed with all asepsis precautions. The count of total germs were carried out after 24 hours of incubation at a temperature of 37 °C. The results were expressed as a decimal logarithm of the colony-forming unit (CFU) per ml of orange juice as defined by the following formula (Berizi et al., 2016):

$$\text{Log 10 CFU / mL} = (\text{CFU} / (V \times FD))\ \text{Log 10}$$

FD is the dilution factor and V is the volume of the sample.

The total flora counted is a major indicator of the microbiological quality of orange juice.

***Data Analysis***

All measurements were carried out in triplicate and the results are expressed as mean ± standard deviation. Data were subjected to F-test analysis of variance using Statistical Analysis System (SAS v. 9.2- Institute Inc., Cary, NC), with complementary Tukey's test with significance level P <0 .05 for the averages.

## Results and Discussion

***Phenolic Composition of Pomegranate Peels***

Determination of total polyphenols and flavonoids showed that pomegranate peels have a content of phenolic compounds of 87.6 mg GAE / g, of which the flavonoids represent 22.00 mg QE / g. These results are similar to those reported by Zaki et al. (2015) who found a content of 113.26 mg GAE / g of phenolic compounds in the PPE methanolic extract, however Viuda-Martos et al. (2012) reported levels of 46.58–54.84 mg GAE / g of PPE. Li et al. (2006) reported also that flavonoids represent only a small part of the polyphenols present in pomegranate peel extract, which was confirmed by our results. Phenolic compounds are secondary metabolites, providing, in addition to their role in the color and maturity of the fruit, an antioxidant function in vitro and in vivo.

***Antioxidant and Antimicrobial Activity***

*Antioxidant activity*

The anti-free radical or antioxidant activity of "*Punica garantum*" peel extract was measured based on DPPH free radical. The latter is an organic radical who will undergo a decrease in its absorbance at 517nm, when it accepts an electron or species of free radicals (Kaneria and Chanda, 2013).

The minimum inhibitory concentration (IC50), thus representing the effective concentration of the antioxidant agent required to lower the initial concentration of DPPH by 50%, was determined from the linear regression between the percentage inhibition versus the concentration of the extract (µg / mL), with a six-point curve and a correlation coefficient $R^2 = 0.991$ (Figure 1). The recorded $IC_{50}$ value is 6.22 µg / mL, indicating a strong antioxidant activity, which is closely correlated with total polyphenol

content (Konsoula, 2016). Indeed, the $IC_{50}$value obtained in the present study is very close to that reported by Kanatt et al. (2010) who showed that the antioxidant activity of pomegranate peel extract with an $IC_{50}$ of 4.9 µg / mL is four times lower than that of BHA ($IC_{50}$ of 21.2 µg / mL). The strong capacity of PPE to scavenge the DPPH radical recorded in the present study indicates that it has a good capacity to give up hydrogen atoms for possible antioxidant power.

*Antimicrobial activity*

Bioactive molecules from the plant have proven to be a good alternative to synthetic molecules used for preserving foodstuffs against spoilage of microbial origin. The antimicrobial activity of PPE against two pathogenic bacteria (*Escherichia coli* and *Bacillus subtilis*) and two fungal strains, isolated from an orange juice that has undergone an alteration (*Saccharomyces cerevisiae* and *Accromyces* sp.) was studied using the aromatogram method, by measuring the inhibition zone. The results obtained are illustrated in Table 1. These results show a variety of inhibitory effects of pomegranate peel extract depending on the bacterial or fungal strain used. It should be noted that the absence of triggered effect of PPE on the bacterium *E.coli* different from *B. subtilis* or the zone inhibition is quite remarkable, with a diameter of 11.8 mm for a minimum inhibitory concentration (MIC) of 37.5µg / mL. Similar results were obtained by Kanatt et al. (2010), who showed that the extract of pomegranate peel has no inhibitory effect on gram-negative bacteria (*E.coli, S. typhimurium, Pseudomonas* spp.), while a total inhibition of gram-positive bacteria was obtained at concentrations order about 0.01%. Other research has also reported that most plant extracts are ineffective against gram-positive bacteria (Oliveira et al., 2008). Unlike bacterial strains, the fungals used in the present study (*S. cerevisiae and Accromyces* sp.) have a lower sensitivity to the PPE extract, with an inhibition zone diameter about 17.5 mm, for a minimum inhibitory concentration of 150 µg / mL. These results are consistent with those reported by Kanoun et al. (2014) who showed that PPE extract inhibits the proliferation of *Rhodotorula* sp., with an inhibition zone of 29.5 ± 0.5 mm for minimal inhibitory concentrations about 0.195≤MIC≤0.39 mg/mL. Indeed, the observed antimicrobial activity is due to the fact that natural plant extracts contain a spectrum of phenolic compounds, whose mechanism of action against microorganisms is postulated as being due to disruption of the cell membrane.

***Effect of pomegranate peel extract on the shelf life of orange juice***

The use of plant extracts as substitutes for synthetic chemical preservatives has become one of the concerns of the agri-food sector, due to the great popularity of organic foods. The effect of pomegranate peel extract on the shelf life of orange juice at concentrations of 0.01, 0.02, 0.03, and 0.04 mg of extract/mL of juice was examined, by monitoring the physicochemical (pH, titratable acidity, TSS, Cloud value and browning index) and microbiological (total microbial load) parameters for 18 days at room temperature (~ 25° C). The results obtained after data statistical analysis are collated in Table 2.

***Effect on pH and Total soluble solid***

The pH and TSS values of the control samples were 3.76 ± 0.18 and 6.813 ± 0.008, respectively. The addition of the phenolic extract of *P. granatum* to orange juice has no significant effect on pH and total soluble solids (TSS), regardless of the dose and storage period (Table 2). The lack of observed effect is probably due to the low dose of PPE used. Contrary to our results, Trigo et al. (2020) observed an increase in pH and TSS following the addition of PPE (5 mg extract/mL) to carrot juice, but no significant variation was observed during the storage period (42 days). In a study examined the use of banana peel extract (BPE) as a preservative for orange juice, Ortiz et al. (2016) reported that the addition of BPE had no significant effect on pH and TSS.

***Effect on Titratable Acidity***

According to Table 2, the addition of PPE to orange juice at different doses has a significant effect on titratable acidity ($P<0.05$). Indeed, average acidities of 1.46 ± 0.18b (negative control), 1.52 ± 0.19 (positive control), 1.28 ± 0.11f (D1), 1.33 (D2) and 1.31 ± 0.11d g of citric acid / 100 ml of juice (D3 and D4) were recorded during the storage period (Figure 1). This difference in acidity between the controls and the samples to which doses of PPE were added is probably due to the blocking of sugar conversion into lactic acid by inhibition of microbial activity using PPE, inducing thus a decrease in titratable acidity (Al-Zoreky, 2009). Similar results have been reported by Sandhya et al. (2018), who showed that the addition of PPE powder to cottage cheese induces a decrease in titratable acidity.

Table 1. Inhibition zone Diameter (mm) of different microbial strains tested

| Species | Concentration (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | 300 | 150 | 75 | 37.5 | 18.75 | 9.37 |
| *Escherichia coli* | 0 | 0 | 0 | 0 | 0 | 0 |
| *Bacillus subtilis* | 28.9 | 26.7 | 24.5 | 11.8 | 0 | 0 |
| *Saccharomyces cerevisiae* | 25.0 | 17.5 | 0 | 0 | 0 | 0 |
| *Accromyces* sp. | 25.0 | 17.5 | 0 | 0 | 0 | 0 |

Table 2. Effect of adding the phenolic extract of "*Punica granatum*" on the various physicochemical parameters (pH, acidity, TSS, browning index, and Cloud value) of orange juice.

| Sample | pH | Acidity | TSS | Browning index | Cloud value |
|---|---|---|---|---|---|
| Test | $3.76 \pm 0.18^{a}$ | $2.18 \pm 0.31^{b}$ | $6.813 \pm 0.008^{c}$ | $0.397 \pm 0.093^{a}$ | $0.198 \pm 0.012^{bc}$ |
| Control (+) | $3.52 \pm 0.22^{c}$ | $1.61 \pm 0.06^{a}$ | $6.813 \pm 0.010^{b}$ | $0.234 \pm 0.065^{f}$ | $0.203 \pm 0.014^{b}$ |
| $D_1$(0,01mg/ml) | $3.60 \pm 0.12^{b}$ | $1.33 \pm 0.08^{c}$ | $7.470 \pm 0.012^{b}$ | $0.254 \pm 0.053^{d}$ | $0.202 \pm 0.091^{b}$ |
| $D_2$(0,02mg/ml) | $3.64 \pm 0.18^{ab}$ | $1.28 \pm 0.11^{f}$ | $7.486 \pm 0.008^{b}$ | $0.245 \pm 0.075^{e}$ | $0.187 \pm 0.011^{c}$ |
| $D_3$(0,03mg /ml) | $3.64 \pm 0.16^{ab}$ | $1.31 \pm 0.11^{d}$ | $7.544 \pm 0.009^{a}$ | $0.286 \pm 0.032^{b}$ | $0.170 \pm 0.094^{d}$ |
| $D_4$(0,07mg/ml) | $3.61 \pm 0.13^{ab}$ | $1.31 \pm 0.09^{e}$ | $7.456 \pm 0.011^{a}$ | $0.263 \pm 0.058^{c}$ | $0.227 \pm 0.082^{a}$ |

*Data are means ± standard deviation. The means with the same letter in a column are not significantly different (Tukey's test, $P<0.05$), with 7 samples of orange juice for each dose.

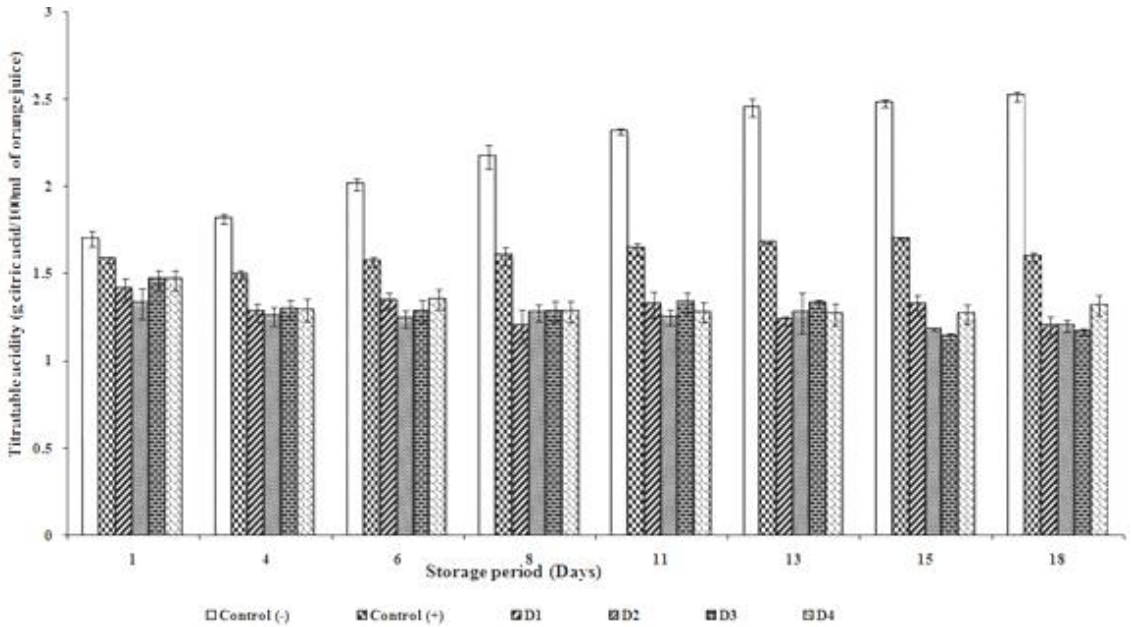


Figure 1. Effect of PPE extract on the titratable acidity of natural orange juice

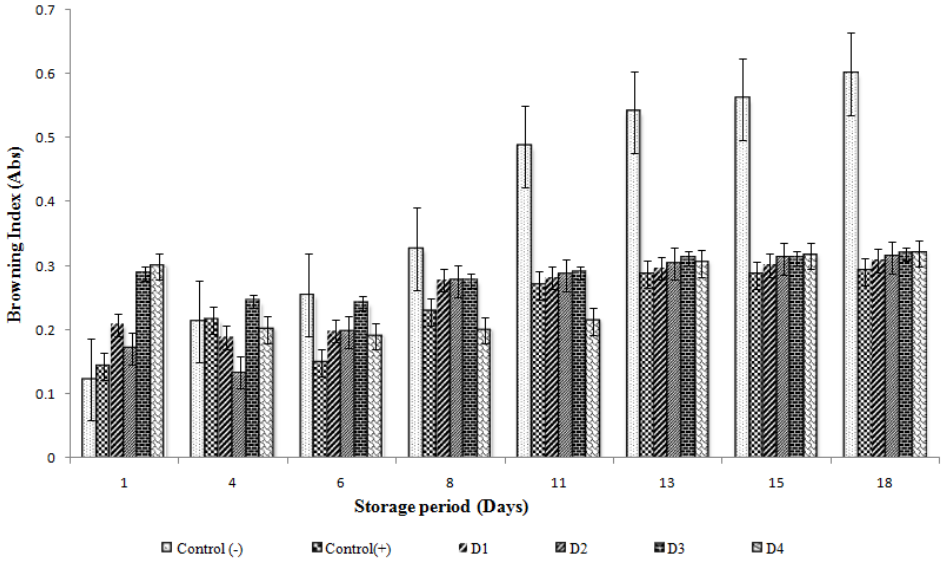


Figure 2. Effect of PPE on the browning index of orange juice.

***Effect on the Browning Index***

The Browning Index is a parameter used to monitor the non-enzymatic browning of fruit juices. From table 2, the browning index increased significantly ($P<0.05$) during the storage period for the control with a mean absorbance of $0.397 \pm 0.093$, while, for juice samples with chemical conservators (citric acid and sodium benzoate) and PPE, the browning index slightly increased during the storage period, with browning indices expressed in absorbance at 420nm of $0.234 \pm 0.065$f, $0.254 \pm 0.053$d, $0.245 \pm 0.075$e, $0.286 \pm 0.032$b and $0.263 \pm 0.058$c for the positive control, D1, D2, D3, and D4 respectively. The observed difference (Figure 2) is probably due to the action of citric acid and PPE against the degradation of anthocyanins of ascorbic acid, and the Maillard reaction which causes an increase in the browning index (Dorris et al., 2018; Johnson et al., 1995). The Browning index was significantly influenced by the addition of PPE ($P<0.05$) as well as chemical conservators. Non-enzymatic browning is a process leading to the degradation of organoleptic and sensory qualities in the majority of fruits; its inhibition thus contributes to an increase in shelf life.

***Effect On Turbidity***

The cloud value of a fruit juice corresponds to the absorbance measured at 660 nm, it is linked to the presence of proteins, pectin, cellulose, hemicellulose, and other microscopic particles. Figure 3 illustrates the change in the cloudiness index of orange juice during the storage period at different doses of PPE. Shelf life significantly influenced juice turbidity without kinetic order ($P<0.05$), while the addition of PPE had no significant effect on juice cloudiness. This can be explained by the fact that PPE does not affect the activity of Pectin Methyl Esterase (PME), de-esterification of methoxylated pectin increased due to the strong activity of PME**.** Iftikhar et al. (2013) reported that the turbidity of orange juice decreased with PME activity, thus inducing a decrease in the cloud value which is considered a major sensory quality defect in citrus juices.

***Effect On Microbiological Quality***

The microbiological quality of the juices supplemented with PPE was determined by monitoring the evolution of aerobic mesophilic germs during the storage period considered (Figure 4).

During the 18 days of storage, the cell concentration of the samples studied gradually increased. However, the concentration of mesophilic aerobic germs in the negative and positive control samples increased rapidly and exceeded the microbiological limit ($10^5$ CFU / mL) after 4 days.

The active samples showed a slow increase in aerobic mesophilic organisms compared to controls, with an extension of the microbiological validity period of 2 days, regardless of the concentration of PPE used, with a significantly difference ($P<0.05$) as shown in Table 3. These results suggest that PPE allows a slowing of microbial growth. Similar results have been reported by Panza et al. (2021), which showed that pomegranate peel powder added to breaded fish fingers, slowed the growth of mesophilic and psychrotrophic germs, compared to control during the storage period. PPE has also shown antimicrobial activity when incorporated into apple juice, carrot juice, and dealcoholized wine (Altunkaya et al., 2013; Trigo et al., 2020; Tárrega et al., 2013). According to the scientific literature, tannins act as an antimicrobial agent by inhibiting enzyme activity, immobilizing substrates such as minerals, vitamins, and organic elements, making them unavailable to microorganisms. Thus, the absorption of phenols by the cell causes a rupture of the wall and consequently a disruption of membrane function and cell exchange (Akhtar et al., 2015; Ismail et al., 2012).

## Conclusion

In the present study, the preservative power (antioxidant and antimicrobial) of pomegranate peel extract (PPE) was evaluated in vitro (on DPPH radical and microbial strains) and in vivo (on a fresh orange juice that has not been pretreated) in order to test its effectiveness as a substitute for synthetic chemical preservatives. The results of in vitro tests revealed high antioxidant and antimicrobial activity, with an $IC_{50}$ of 6.22 µg / mL and variable zones of inhibition depending on the bacterial or fungal strain considered. Likewise, in vivo tests have shown very highly significant effects between the physicochemical and microbiological parameters of orange juice and the dose of PPE. Indeed, the addition of PPE to fresh orange juice has no effect on the pH, TSS, and cloud value, while it has a positive and significant influence ($p<0.05$) on browning index and titratable acidity, with a reduction in browning reactions and acidity compared to controls. An effect on the microbial load was also observed with an extension of the shelf life of fresh juice by 2 days compared to the controls. Consequently, it can be concluded that pomegranate peels can be reused in the conservation of fresh products and constitute a sustainable way to reduce the harmful effects linked to the use of synthetic products, for a healthy and sustainable diet.

Table 3. Effect of supplementation with *Punica granatum* peel extract on the microbiological quality of orange juice.

| Sample | Log 10 CFU/ml |
|---|---|
| Control (-) | $6.75 \pm 0.027^{a}$ |
| Control (+) | $6.46 \pm 0.109^{b}$ |
| $D_1$ (0.01 mg/mL) | $6.11 \pm 0.180^{c}$ |
| $D_2$ (0.02mg/mL) | $6.12 \pm 0.209^{d}$ |
| $D_3$ (0.03mg /mL) | $6.05 \pm 0.173^{e}$ |
| $D_4$ (0.07mg/mL) | $5.99 \pm 0.104^{f}$ |

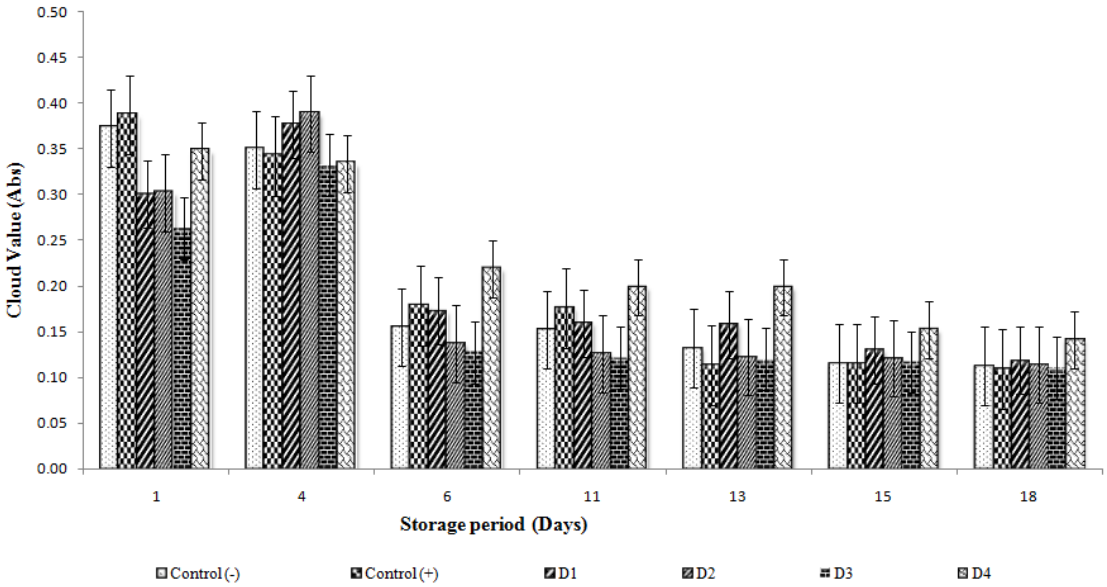


Figure 3. Effect of PPE on the turbidity of orange juice during storage

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