

Original Research Article

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Effect of *Origanum elongatum* essential oil and heating on Pomegranate Juice Quality

Naima El Moussaoui*, El Ouardy Khay, Nadia Amajoud, Nadia Boujida,
Saoulajan Charfi, Nadia Skali Senhaji and Jamal Abrini

University Abdelmalek Essaâdi, Faculty of Sciences, Department of Biology, Laboratory of
Biology and Health, M'hannech II, BP 2121, Tetouan 93002, Morocco

*Corresponding author

ABSTRACT

Keywords

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The aim of present study was to determine the effect of *Origanum elongatum* essential oil on the quality of fresh pomegranate juice. The antimicrobial activity of 0.06% (v/v) of essential oil alone and in combination with heat treatment (70 and 80°C) was evaluated for 30 seconds. Meanwhile, the content of total sugars and pH was recorded at 20°C for 16th day during conservation. The concentration of 0.06% of essential oil showed a significant effect on microbial growth and chemical variation. Furthermore, it was found that the combined treatment with heat increased the effect of essential oil on microbial viability, and consequently improved the juice conservation process while preserving nutritional and organoleptic qualities.

Introduction

The pomegranate (*Punica granatum* L.), one of the oldest edible fruits, has attracted great attention for its health benefits in recent years, due to the attribution of important physiological properties (Rahimi *et al.*, 2012). In general, because of the composition of fruit juices and their lower pH (pH <4) they provide an ideal environment against the deterioration caused by yeasts, molds and lactic acid bacteria that can grow in very acidic environments (Bevilacqua *et al.*, 2011).

Heat pasteurization is the most common method among the various technologies used to preserve fruit juice from deterioration (Patil *et al.*, 2010). However, since this treatment can cause changes in the chemical composition of the juice, to meet the increasing consumer demand and preferences for "like fresh" products with minimal loss of vitamins and flavours further investigations are still needed in the field. Recently, the use of natural plant extracts as alternative to antimicrobial chemical is a growing trend in the food

industry (Perumalla and Hettiarachchy, 2011). Various essential oils and their compounds are tested in fruit juices, either alone or in combination treatments as preservative (Akpomedaye and Ejechi, 1998; Tserennadmid *et al.*, 2011; Tyagi *et al.*, 2013).

In the present work, the chemical analysis of pomegranate juice has been done by measuring pH and by colorimetric assays of total sugars. Then we studied the effect of *Origanum elongatum* essential oil (EO) on the natural flora pomegranate juice, alone and in combination with the effect of temperature, by tracking the changes in pH and total sugars during conservation period.

Materials and Methods

Sample Preparation

In this study, we tested the activity of *O. elongatum* EO extracted from flowering tops (Voucher specimens: INP 1234) and we used fresh pomegranate juice as model aliment. In each sterile flask, 100 ml juice was added. To evaluate the interaction between EO effect and temperature, we studied one concentration (0.06% v/v) of *O. elongatum* EO and two temperatures (70 and 80°C). Each bottle, with or without EO, were subjected to heating in a water bath for 30 seconds. The juice samples were immediately cooled in an ice water bath. The control samples, were prepared with fresh pomegranate juice without heating and/or EO.

Chemical Analysis

pH Measurement

The pH variation in tested pomegranate juice was measured by using a pH meter (HANNA INSTRUMENTS, Romania).

Determination of Total Sugars

The furfural (2-Furancarboxaldehyde formula $C_5H_4O_2$) reaction method applied by Dubois *et al.* (1956) and described by Strickland and Parsons (1968) was used in this study to evaluate the monosaccharide present in food samples. One ml of standard or juice sample was added to 1 ml of the aqueous solution of phenol (50 mg/ml) and 5 ml of concentrated sulfuric acid was quickly added for immediate mixture. Then, the samples were cooled down to room temperature for 30 min, and the absorbance was measured at 490 nm. Distilled water was used as control. The calibration curve was performed from dilutions of the prepared aqueous glucose solution in a concentration range between 0.0125 to 0.125 g/L.

The variations of pH and juice total sugar content during storage were also monitored for each sample at days 0, 5, 10 and 16. The chemical products used in these assays were all from SIGMA-ALDRICH (Steinheim, Allemagne).

Microbiological Analysis

We followed the growth of natural flora in the pomegranate juice samples under different treatments. Thereafter, the treated vials were stored at 20°C until the 16th day and aliquots were taken on days 0, 1, 2, 3, 4, 5, 6, 10 and 16. Serial dilutions were prepared from each aliquot. The enumeration of total mesophilic aerobic flora (FMAT) was evaluated on plate count agar medium. Medium of de Man, Rogosa and Sharpe agar was used to detect lactic acid bacteria. Yeasts and molds were counted on Sabouraud Chloramphenicol Agar medium. All media were supplied by BIOKAR DIAGNOSTICS (Beauvais, France). The reading of the results was

carried out after 72 hours cultivation at 30°C for FMAT and lactic acid bacteria, and between 3 to 5 days for the yeasts and molds.

Statistical Analysis

The statistical analysis was performed using STATISTICA v6 software. The juice experiments as well as the dilutions were repeated twice. The colorimetric assays were performed in triplicate. Results are expressed as mean \pm SD (n = 3). Significant differences were determined using ANOVA test followed by Tukey's test to correct for multiple comparisons. Differences were considered significant at $p < 0.05$.

Results and Discussion

Chemical Property

Chemical analyzes were performed to pomegranate juice. Pomegranate juice presented an acidic pH (3.42 ± 0.02) with high total sugar content (131.913 ± 2.24 g/L).

The pH value of pomegranate juice in this study was in the range of pH values obtained by Hmid *et al.* (2013) (2.85-4.22), Tehranifar *et al.* (2010) (3.16 to 4.09) and Zaouay *et al.* (2012) (2.72-4.24). However, total sugar content of our pomegranate juice was lower than the range content found in Turkish varieties (139.6-160.6 g/L) (Poyrazoğlu *et al.*, 2002).

The differences observed in the analyzed parameters are attributed to different pomegranate varieties studied (genotype), the diversity in the agro-climatic conditions and in the maturity rate (Hasnaoui *et al.*, 2011; Fawole and Opara, 2013).

Variations of juice pH and total sugar

content during storage period were monitored (Figure 1 and 2). During the conservation, control and heat treated only samples, have suffered a significant reduction in total sugar content (135.18-11.30 g/L) ($p < 0.001$; $r = -0.99^*$) and pH (3.43-3) ($r = -0.91^*$) as found by Ezoua *et al.* (2008). The treatment with EO alone or combined with heat showed a small and non-significant variation in sugar content (136.13-127.15g/L) and pH (3.51-3.34) ($p > 0.05$).

In fact, monitoring of pH and total sugars content, during conservation, clearly showed the preserved quality of juice samples treated with 0.06% of EO alone or combined with heat. Meanwhile, in the control samples, pH and total sugars content showed significant decline due to fermentation process conducted by acidophilic microorganisms.

Microbiological Analysis

The various microbiological analyses are presented in figure 3, 4 and 5. The juice without treatment, presented a significant growth of native flora from the 1st day of storage.

In the presence of 0.06% of EO alone, the growth of FMAT was maintained at 2.68 ± 0.28 log CFU/ml until the 5th day. Lactic acid bacteria were undetectable (<10 CFU/ml) in the second day, suggesting a progressive growth that reached 2.87 ± 0.16 log CFU/ml on the 5th day. While the growth of yeasts and molds during the 5 days treatment remained at <2 log CFU/ml and <3 log CFU/ml till day 16 ($p < 0.001$ vs. control). Therefore, yeasts and molds appear to be more sensitive to the EO activity than lactic acid bacteria ($p < 0.05$).

In the literature, there are examples of

application of EO and their components (citral, β -pinene, limonene, linalool, eugenol and α -pinene), alone or combined with other compounds to extend shelf life of fruits or fruit juices (Belletti *et al.*, 2010; Tserennadmid *et al.*, 2011; Castillo *et al.*, 2014).

In the present study, the activity of 0.06% of the EO in the juice was stronger against yeasts and molds ($<3 \log \text{CFU/ml}$) than against lactic acid bacteria ($p < 0.05$), which was consistent with the results found by other authors (López *et al.*, 2007; Bevilacqua *et al.*, 2010).

Figure.1 Evolution of Total Sugar Content in Pomegranate Juice Treated by EO at 20°C and/or Combined with Heat during the Storage Period

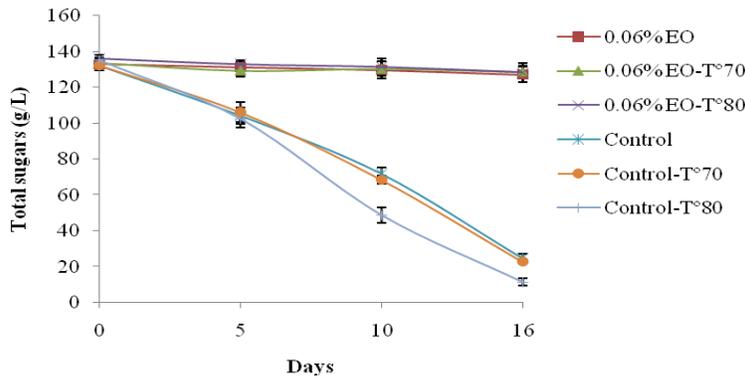


Figure.2 Evolution of pH of Pomegranate Juice Treated with EO at 20°C and/or Combined with Heat During the Storage Period

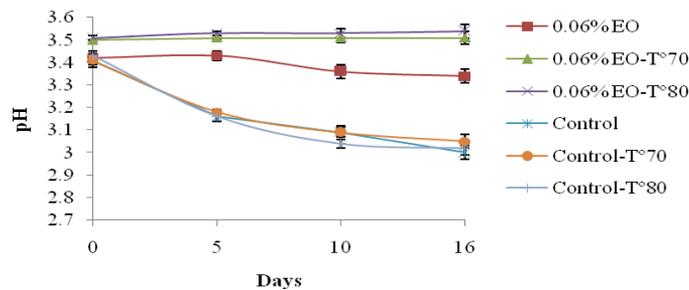


Figure.3 Evolution of FMAT on Pomegranate Juice Processed by EO Combined with Heat during the Storage Period

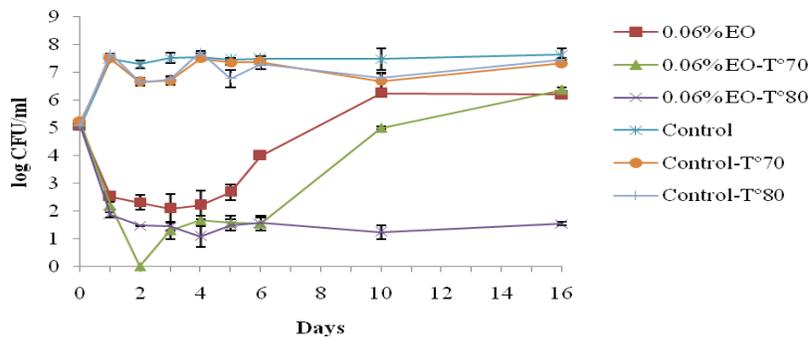


Figure.4 Evolution of Lactic Acid Bacteria on Pomegranate Juice Processed by EO Combined with Heat During the Storage Period

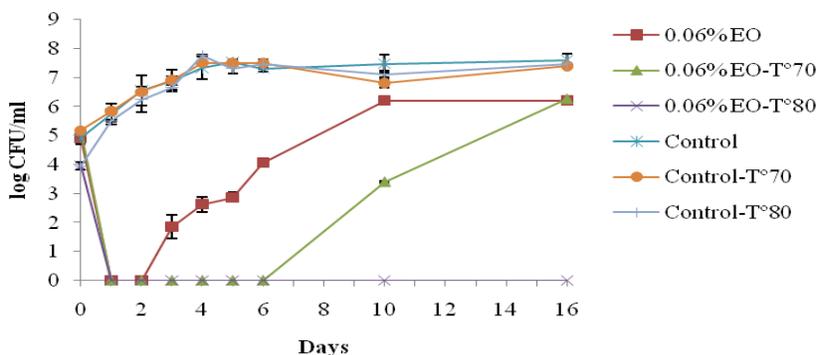
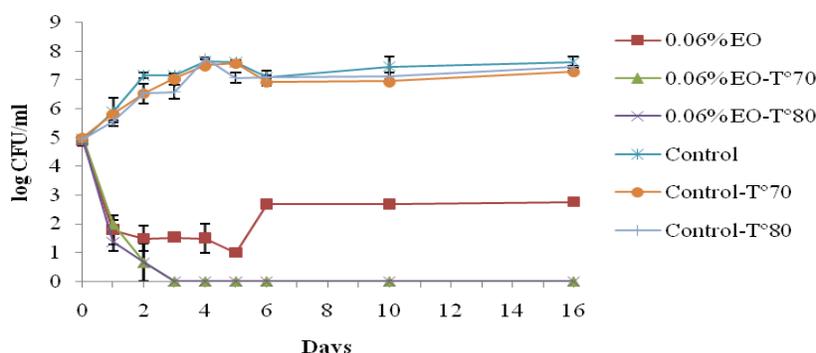


Figure.5 Evolution of Yeasts and Molds on Pomegranate Juice Processed by EO Combined with Heat During the Storage Period



The heating alone, at 70 and 80°C for 30 seconds, had small and non-significant effect on the growth of lactic acid bacteria (0.22 ± 0.23 ; 0.14 ± 0.19 log CFU/ml), yeasts and molds (0.35 ± 0.21 ; 0.19 ± 0.18 log CFU/ml) and FMAT (0.31 ± 0.19 ; 0.18 ± 0.26 log CFU/ml) contained in pomegranate juice ($p > 0.05$ vs control). While, heat (80°C) treatment combined with EO declined the growth of FMAT from the 1st day to <2 log CFU/ml ($p < 0.01$ vs control). However, heat (70°C) treatment combined with EO showed an increase in the growth of FMAT to 6.37 ± 0.05 log CFU/ml on day 16 ($p < 0.05$ vs control). Furthermore, lactic acid bacteria were undetectable from the 1st day in the juice treated by EO and 80°C, but the treatment

with a combination of EO and 70°C allowed lactic acid bacteria to reach 6.27 ± 0.08 log CFU/ml at day 16 ($p < 0.001$ vs control). Likewise, yeast and mold growth was reduced from the 2nd day (<1 log CFU/ml, $p < 0.001$ vs control) by the combined treatment of juice with EO and heating both at 80°C and 70°C and remain undetectable during the whole storage period.

Data from the present study showed that the treatment with heat alone for 30 seconds either at 70 or 80°C has no effect on the initial load of yeasts and molds or FMAT content in pomegranate juice. Only a small variation in the growth of lactic acid bacteria was observed after heat treatment. In fact, treatments with 80°C for 30, 60 and 90

seconds are almost ineffective for preserving the juice spoilage by yeasts (Tyagi *et al.*, 2013).

The combination of EO and heat at 70°C minimized juice fermentation for one week but did not affect the yeasts and molds. However a combination of EO and 80°C treatment was sufficient to inhibit the natural flora tested since the 2nd day. Similarly, Tyagi *et al.* (2013) found that a combination of heat treatment (80°C for 30, 60 and 90 seconds) and the mint EO completely inhibits the growth of *Saccharomyces cerevisiae* after two days storage of fruit juices mixture at room temperature.

From the results, synergistic effect clearly appeared by comparing the juice treated with 0.06% of EO alone and combined with the heat. This combination of EO and heat treatment seemed to reduce more the microbial viability (Tyagi *et al.*, 2013; Belletti *et al.*, 2010).

Indeed, temperatures above 55°C increase the vapour pressure of the volatile compounds, and consequently enhance their ability to be dissolved in the plasma membrane of yeast and thus increase their bioactivity (Lanciotti *et al.*, 2004).

As made obvious by previous studies, EO vapours have a greater effect than the corresponding oil on yeast (Tyagi and Malik, 2010). The addition of volatile compounds (eugenol, thymol, menthol) within packets is effective in reducing the proliferation of microorganisms in the table grapes, the effect being greater for yeasts and molds than for aerobic mesophilic bacteria (Valverde *et al.*, 2005) and is consistent with our results obtained with the combined treatment at 70°C. Though storage of pomegranate juice at high temperatures can modify the nature of the juice that is

both acid and sweet, EO seems to have a significant effect on the growth of strains, and the deterioration of juice.

In general, the antimicrobial activity of EO increases as the pH decreases. The antibacterial efficacy of oregano and thyme EO was shown by Gutierrez *et al.* (2009) at a pH 5. In fact, the susceptibility of bacteria to EO appears to increase with low pH values, and the hydrophobicity of EO increases at a low pH, which consequently makes it easy to dissolve in lipids of target bacteria cell membranes (Juven *et al.*, 1994).

However, Tserennadmid *et al.* (2011) showed that the inhibitory effect of some anti-yeast EO (clary sage, juniper, lemon and marjoram) was maximal at pH 7, and is also efficient in the acidic media, suggesting that the ionized forms of EO components did not play a major role for acidophilic yeasts. In addition, the presence of high concentrations of carbohydrates (5.8 or 11.6%) has no negative impact on the efficiency of EO (Gutierrez *et al.*, 2009).

In conclusion, the nature of pomegranate juice did not affect the activity of EO alone or combined with heat. The simple treatment with 0.06% of EO showed a significant effect on the growth of micro-organisms. The combined treatment with EO and heat enhanced their negative effect on the viability of the micro-organisms tested. This observed synergistic effect allowed the use of a low EO concentration for fruit juices conservation to keep their nutritional and organoleptic properties appreciable.

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