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Effect of clarification on some characteristics of pomegranate juice processed by two different methods during storage

Efeito da clarificação sobre algumas características do suco de romã processado por dois métodos diferentes durante o armazenamento

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ABSTRACT

This study aimed to assess the effectiveness of two clarifying procedures and their effects on some properties in thermally or microwave-pasteurized pomegranate juices. The experiment consisted in combining pectinase and protease as well as chitosan and gelatin once they were being stored in the refrigerator. The experiment consisted in three parts, being the first one a fresh juice without any clarification treatment, whereas, the second one was the fresh juice treated with clarifying agent consisted of pectinase and protease mixture ratio (2:1) at 0.75 v/v,and 50 °C for 20 min. Finally, the third one was fresh juice treated with chitosan and gelatin mixture at 0.4 and 0.8) g/L, respectively, at 50 °C for (20) min. The pasteurization of all three experiments was done by using two techniques, i.e., one with thermal water bath at 85 °C for two min and the microwave at 400 Watts for two min. All pomegranate juice bottles were stored at 4 °C for three months. The results showed a significant effect of the clarification method variable on the properties studied, especially turbidity, polyphenol and anthocyanin values. Moreover, the juice clarified with the enzymatic clarification method had better characteristics than the traditional ones during storage, what has therefore a better commercial appeal. The area of significance was founded with the use of traditional clarification with concentration at (0.4 and 0.8) g/l, and microwave pasteurization with 400 watts and zero month of storage at 4 °C, respectively, which is provided a minimum turbidity value.

KEYWORDS: pomegranate juice; clarifiers; microwave.

RESUMO

Este estudo teve como objetivo avaliar a efetividade de dois procedimentos esclarecedores e seus efeitos sobre algumas propriedades em sucos de romã termicamente ou pasteurizados por micro-ondas. O experimento consistiu em combinar pectinase e protease, bem como quitosana e gelatina, uma vez que estavam sendo armazenadas na geladeira. O experimento consistiu em três partes, sendo a primeira um suco fresco sem qualquer tratamento de clarificação, enquanto a segunda foi o suco fresco tratado com agente clarificante consistiu na proporção de mistura de pectinase e protease (2:1) a 0,75 v/v, e 50°C por 20 min. Finalmente, o terceiro foi suco fresco tratado com mistura de quitosana e gelatina a 0,4 e 0,8) g/L, respectivamente, a 50 °C por (20) min. A pasteurização dos três experimentos foi realizada utilizando-se duas técnicas, ou seja, uma com banho de água termal a 85 °C por dois min e a micro-ondas a 400 Watts por dois min. Todas as garrafas de suco de romã foram armazenadas a 4 °C por três meses. Os resultados mostraram um efeito significativo da variável método de clarificação sobre as propriedades estudadas, especialmente os valores de turbidez, polifenóis e antocianinas. Além disso, o suco clarificado com o método de clarificação enzimática apresentou melhores características do que os tradicionais durante o armazenamento, o que tem, portanto, um melhor apelo comercial. A área de significância foi fundada com o uso de clarificação tradicional com concentração a (0,4 e 0,8) g/l, e pasteurização por micro-ondas com 400 watts e zero mês de armazenamento a 4 °C, respectivamente, o que proporciona um valor mínimo de turbidez.

PALAVRAS-CHAVE: suco de romã; Clarificadores; microondas.

INTRODUCTION

Pomegranate fruit and its juice have potential health beneficial properties well-established by scientific researches (CANO-LAMADRID et al. 2019, KUJAWSKA et al. 2019, HEGAZI et al. 2021). Therefore, the demand for pomegranate in the world showed a considerable increase, especially for pomegranate juice consumption (GIMÉNEZ-BASTIDA et al. 2021). The consumption of pomegranate juice became popular, which may be attributable to its antioxidative characteristics that based largely on their content of phenolic compounds, such as catechins, ellagic tannins, and anthocyanins (VIUDA-MARTOS et al. 2010).

Pomegranate fruit contains pectin and other carbohydrates, which tends to cause turbidity in the fresh pomegranate juice, and hinder the marketability for the fruit and vegetable processors (VAILLANT et al. 2001). The reasons are due to some chemical components of the pomegranate juice, especially anthocyanins and tannins, with great antioxidative activity (CONIDI et al. 2020). But at the same time, the two components contribute to the formation of cloudiness during the juice storage, which often negatively affect the juice quality and, indirectly, on the market. Therefore, clarification is a necessary step in pomegranate juice processing to remove particles which cause the development of turbidity and settling and thus prevent haze formation in the juice during storage (CASSANO et al. 2011, MIRZAAGHAEI et al. 2016). The storage conditions such as temperature and time were selected based on other published papers. Currently, many clarification procedures can be applied by different techniques such as filtration, centrifugation, and some agents (e.g., chitosan, gelatin, pectinase, protease) or a combination of these techniques (PINELO et al. 2010, YOUSEFNEZHAD et al. 2017). In general, the cost of the clarification ways of cloudy fruit juices were differently depending on some factors such as type of clarification agents used and if the fruit juice production is applicable on small or large scale of food plants.

The influencing factors of fruit juice consumption largely depending on consumer preferences of high quality products. Most of the fruit juice clarification studies aimed to produce different juices with high degree of quality and low level of tubidity. The local consumers always trying to find fruit juices with high rank of quality. Our research is one of the studies carried out to meet with all above objectives and market requirements.

The approach for using clarification process has been determined under various conditions with different types of fruit juices such as red grape juice (DIBLAN & ÖZKAN 2021), sugarcane juice (MENG et al. 2021), pear juice (AMOBONYE et al. 2022), black mulberry juice (ASKIN et al. 2022), as well as cashew apple juice (ALUKO et al. 2023). The main causes of cloudiness in juice types are phenolics, pectins, and proteins, therefore, the target of these studies was removal of turbidity causes. A lower turbidity and higher retention of quality properties of clarified fruit juices were resulted.

However, selection of effective clarification method with each fruit juice type is challenge for food industry since amounts of turbidity causes are different among various fruit juices.

Therefore, the goal of this research is to evaluate the efficiency of two different clarification combination agents (pectinase and protease, chitosan and gelatin) on total soluble solids, total titratable acidity, pH, turbidity, polyphenol content and total anthocyanin content in each thermally pasteurized or microwave pasteurized pomegranate juices during storage at 4 °C for three months.

MATERIAL AND METHODS

The pomegranate fruits (*Punica granatum* L.) used were supplied from a major market in Mosul, Iraq, and were stored at 4 °C until they were used to experiment. The amount of 6 -7 kg of fresh pomegranate fruits were used.

Pectinase preparation from Aspergillus niger with activity ≥1 units/mg was procured from the DSM company (Barcelona, Spain), while protease preparation with activity ≥ 0.6 units/mg was supplied from Sigma Aldrich (St Louis, MO, USA). The two enzyme preparations were selected based on a preliminary experiments. All other chemicals used for analytical purposes were supplied from Sigma Aldrich (St Louis, MO, USA), and were of analytical grade.

The Pomegranates were selected based on uniform size and appearance, washed manually with water to remove any contaminated surface particles, and drained. Then, pomegranate fruits were peeled and the rinds covering the seeds were removed manually by hands. After, the juice was extracted from the arils by a laboratory scale type press in a University of Mosul pilot plant.

The pomegranate juice samples were divided into three groups for clarification treatments. The first group consisted of samples of pomegranate juice with no added clarification agents, and then it was divided into two subgroups. The first subgroup was pasteurized with a water bath at 85 °C for two min and labeled as (NCTPPJ). The second subgroup was pasteurized by using microwave oven at 400 Watts for two min, and labeled as (NCMPPJ).

The second group subsist of samples of pomegranate juice clarified with pectinase and protease mixture solution with ratio 2:1 and concentration at 0.75 v/v %. The incubation temperature was performed at

50 °C and time for clarification was 20. The second group was divided into two subgroups. The first subgroup was pasteurized with water bath at 85 °C for two min and labeled as (ECTPPJ). The second subgroup was pasteurized in microwave oven at 400 Watts for two min, and labeled as (ECMPPJ).

Finally, the third group abide samples of pomegranate juice clarified with chitosan and gelatin solution in combination with each other at two concentrations (i.e., 0.4, 0.8 g/L). The incubation temperature and time for clarification was performed at 50 °C for 20 min. Then, two subgroups were proposed, being the first subgroup pasteurized in water bath at 85 °C for two min and labeled as (TCTPPJ) and the second subgroup was pasteurized with microwave oven at 400 Watts for two min, and labeled as (TCMPPJ), and the meaning of abbreviations used in this research are shown in the following table.

Table 1. Abbreviations used in this research and their meanings.

Number of treatment	Abbreviation used	Its meaning
1	NCTPPJ	Non-Clarified Thermal Pasteurized Pomegranate Juice
2	NCMPPJ	Non-clarified microwave pasteurized pomegranate juice
3	ECTPPJ	Enzymatic Clarified Thermal Pasteurized Pomegranate Juice
4	ECMPPJ	Enzymatic Clarified Microwave Pasteurized Pomegranate Juice
5	TCTPPJ	Traditional Clarified Thermal Pasteurized Pomegranate Juice
6	TCMPPJ	Traditional Clarified Microwave Pasteurized Pomegranate Juice

It is important to mention that preliminary tests obtained the above-mentioned clarification conditions. At the end of enzymatic clarification treatments, the enzyme was inactivated by heating the mixture at 85 °C for two min. Following that, juice samples were packaged in 72 glass bottles with 100 ml capacity and immediately cooled to refrigeration temperature in a cold-water bath. Interestingly, all juice samples were stored at 4 °C for three monthswhich were selected based on previous literature.

The glass bottles were then submitted to the total Soluble solids (TSS) content using a refractometer (digital handheld L.002-R.2, Germany) and expressed as Brix values.

Titratable acidity (TA) measurements were made as outlined by CHATTERJEE et al. (2004) and the results are calculated and expressed as g.anhydrous citric acid /100 ml juice. Complementary, pH values of the pomegranate juice were performed with a pH meter (WTW 530) at 20 °C.

The turbidity values as nephelometric turbidity units (NTU) of the pomegranate juice samples were measured with a turbidity meter (Hanna instrument LP 2000) according to the methodology proposed by TAŞTAN & BAYSAL (2017).

The polyphenolic content of the juice samples was assessed using Folin-Ciocalteu reagent procedure and spectrophotometer at 765 nm as mentioned by SHAH et al. (2015).

The pH differential method was used to measure the total anthocyanin content in the juice (TURFAN et al. 2011), and the results were expressed as mg/l.

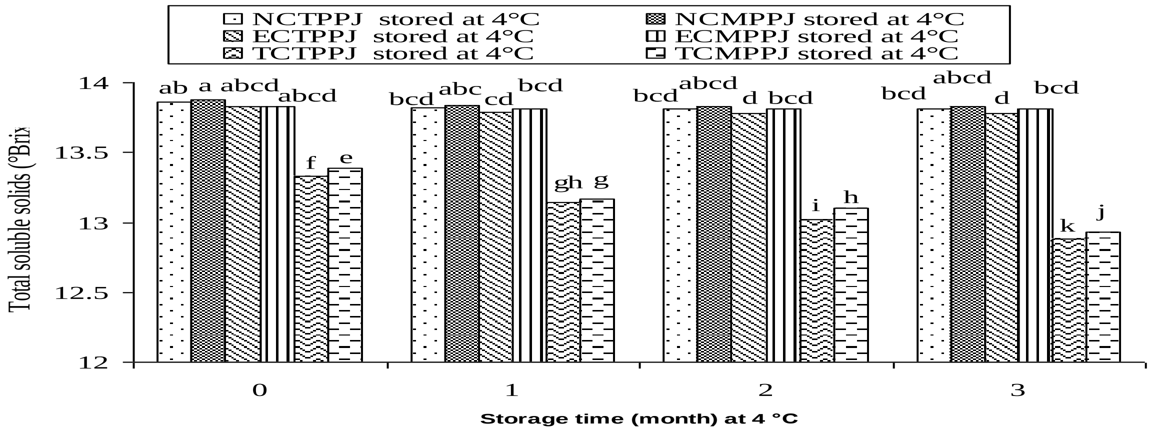
The determinations of total soluble solids (TSS), titratable acidity (TA), pH, turbidity, anthocyanin content, total phenolic content (TPC), were conducted for all pomegranate juice groups after its pasteurization by both methods, and after its storage each month interval at 4 °C for three months. This period was choosen because it is largely applied in the market and this is also the timeframe in which the fruits characteristics are maintained (is this right? I am just trying to find elements to justify the period of time here).

Statistical analysis

In order to answer the stated goal, the main variables considered here were clarification methods (two groups and two subgroups each), pasteurization techniques (thermal and microwave), and storage months (three months). All statistical analyses were run using SAS software (SAS institute, Cary, version 9.4). The Duncan's test (SOKAL & ROHLF 1995) was used to show the statistical differences between means of variables.

RESULTS AND DISCUSSION

Brix degrees of the total soluble solids (TSS) of pomegranate juices treated with two clarification methods compared with non clarified juices are presented in Figure 1. TSS value (13.83°Brix for both ECTPPJ and ECMPPJ was nonsignificant lower than that of the NCTPPJ and NCMPPJ (13.86 and 13.88) °Brix, while TCTPPJ and TCMPPJ have total soluble solids (13.33 and 13.39) significantly lower (*P*<0.05) than that NCTPPJ and NCMPPJ, respectively, during storage.



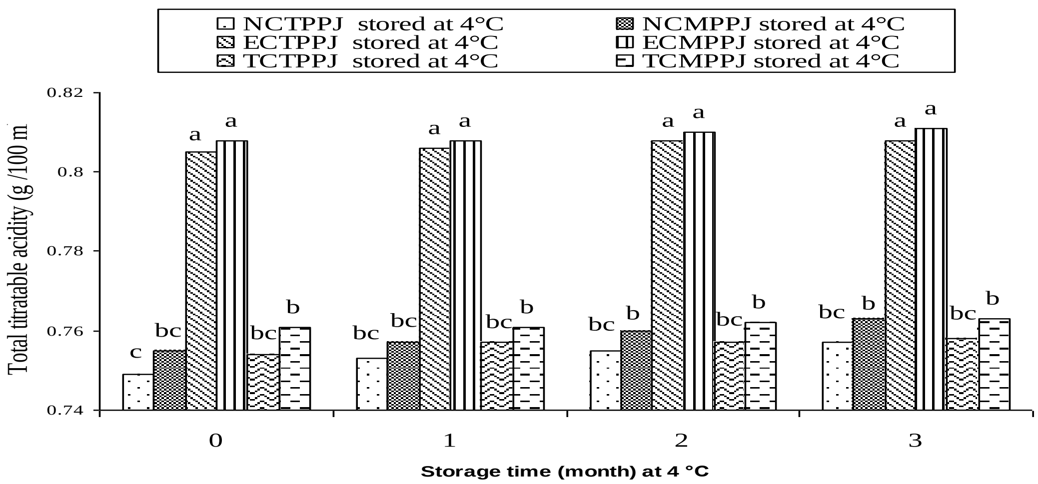
See the meaning of each abbreviation in Table 1. The values are obtained as average of three determinations of juice samples. For each time of storage, data with different lowercase letters refer to significant differences at (p<0.05).

Figure 1. Effects of clarification treatment, pasteurization method and storage time on total soluble solids (°Brix) of pomegranate juice.

In both thermally and microwaved pasteurized juices, the juice samples treated with enzymes had TSS content that did significantly differ each other (p<0.05) in comparison with those treated with a combination of chitosan and gelatin. The results of Figure 1 also showed that the TSS of Juice samples labeled as TCMPPJ were significantly higher than those labeled as TCTPPJ with scores of 13.39 and 13.33 °Brix, respectively.

The decreasing in the TSS content of NCTPPJ, NCMPPJ, ECTPPJ, ECMPPJ, TCTPPJ, and TCMPPJ pomegranate juices continued until the third month of storage at 4 °C. In our study, the significant changes in the TSS of the traditional clarified pomegranate juice during storage were different from that reported by ALUKO et al. (2023). The authors reported no significant changes after juice clarification, and these differences would be due to that different clarification conditions. In summary, the results agreed with previous studies carried out with different treatments and fruit juices (ERKAN-KOÇ et al. 2015, DERELI et al. 2015).

Changes in the total titratable acidity values of pomegranate juice samples (g/100 ml) are shown in Figure 2. After clarification and pasteurization treatments, total acidity values increased to 0.805, 0.808, 0.754 and 0.76 g/100 ml. in pomegranate juices labeled as NCTPPJ-TCMPPJ, respectively, in comparison with nonclarified and pasteurized samples at 0th week of storage. Different from the effect of enzymatic clarification agents, traditional clarification agents (i.e., chitosan and gelatin) did not affect titratable acidity in pomegranate juices.



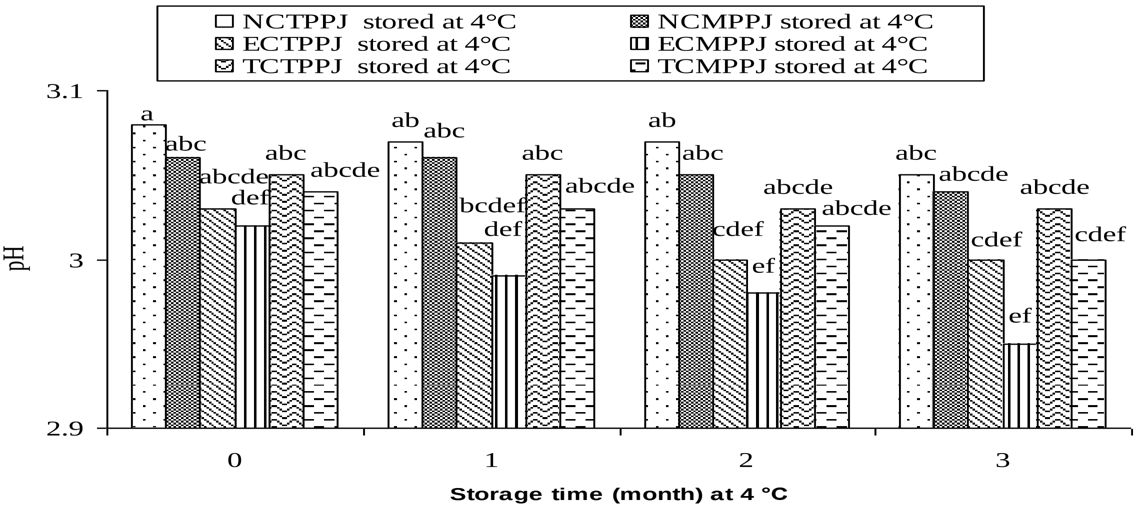
See the meaning of each abbreviation in Table 1. The values are obtained as average of three determinations of juice samples. For each time of storage, data with different lowercase letters refer to significant differences at (p<0.05).

Figure 2. Effects of clarification treatment, pasteurization method and storage time on total titratable acidity (g anhydrous citric acid /100 ml) of pomegranate juice.

As the storage time increased, acidity values of juices increased during storage, and these values with enzymatically treated samples were higher than those of traditionally treated ones, as seen in Figure 2. Similar findings were indicated by TURFAN et al. (2012), such that a slight increase for the total titratable acidity of the pomegranate juice sample was seen after their clarification with traditional agents and storage.

In general, the obtained findings agree with DERELI et al. (2015), DAVARCI et al. (2019), WANG et al. (2022) and ALUKO et al. (2023).

Figure 3 shows the pH of pomegranate juices treated with enzymatic mixture composed of pectinase and protease (ECTPPJ and ECMPPJ) and with a mixture consisting of chitosan and gelatin (TCTPPJ and TCMPPJ). Clarification processes led to insignificant reductions in pH values of juice samples, except juice samples that were clarified by enzyme mixture and pasteurized with microwave oven during storage for three months at 4 °C. Pomegranate juice samples had a minimum pH in TCTPPJ group than NCTPPJ, NCMPPJ, ECTPPJ, ECMPPJ, and TCMPPJ groups during storage at 4 °C for three months. Similarly, the different effects of various clarifiers on pH values were displayed in numerous studies (GHOSH et al. (2016), PARFAIT et al. (2022), and YILDIZ et al. (2022)).



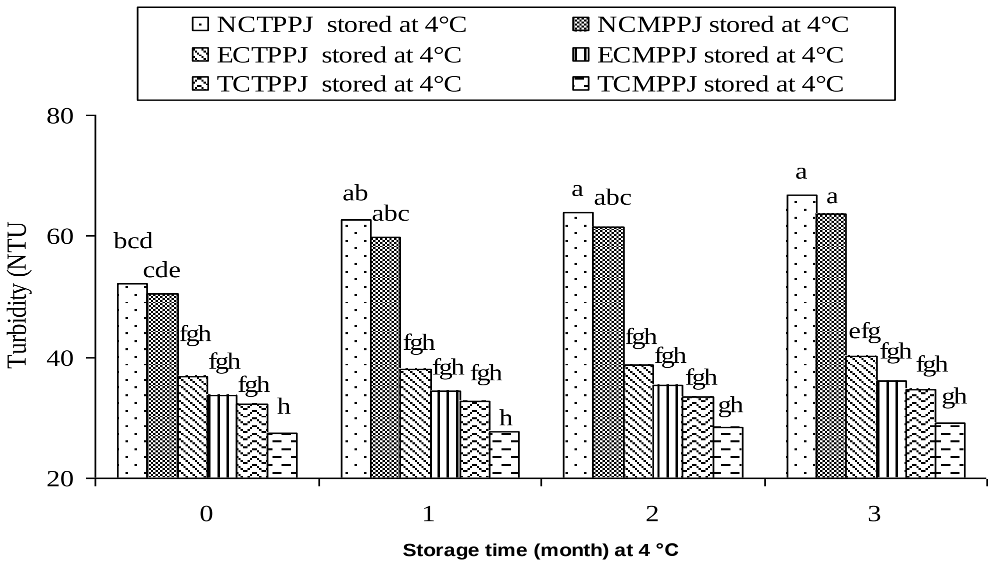
See the meaning of each abbreviation in Table 1. The values are obtained as average of three determinations of juice samples. For each time of storage, data with different lowercase letters refer to significant differences at (p<0.05).

Figure 3. Effects of clarification treatment, pasteurization method and storage time on pH of pomegranate juice.

Turbidity measurement is used to determine whether or not the applied clarification process is efficient. The study showed that the turbidity values of NCTPPJ, ECTPPJ and TCTPPJ were ranged from 52.23 to 66.91, 32.17 to 34.69, and 36.90 to 40.10 NTU, respectively, during storage time at 4 °C. The NCMPPJ, ECMPPJ, and TCMPPJ had turbidity values ranging from 50.56 to 63.70, 27.35 to 29.07, and 33.68 to 36.00 NTU, respectively, for juice samples stored at 4 °C.

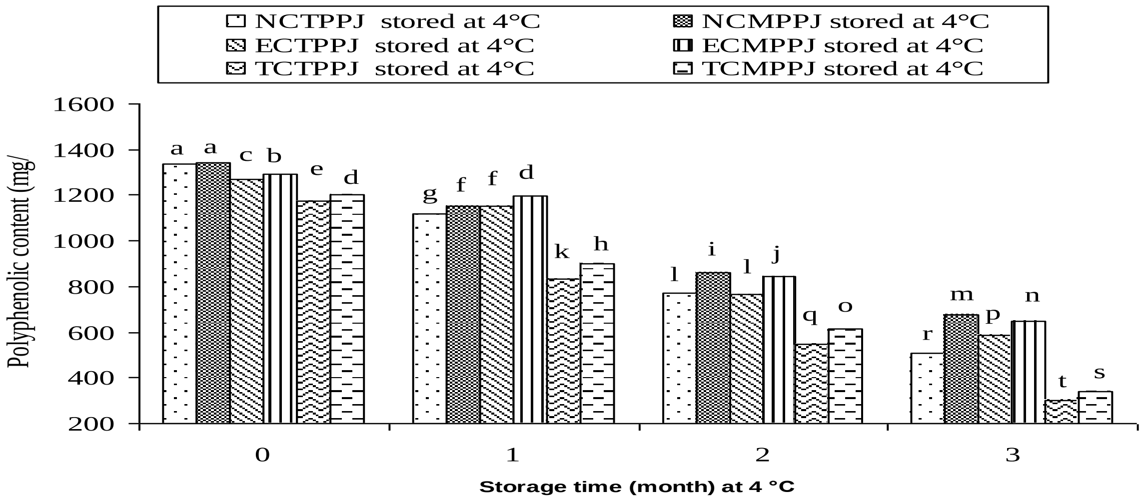
The results presented in Figure 4 showed no effects related to the pasteurization process type on turbidity values for all juice samples. According to the results shown in Figure 4, Pomegranate juice samples had a minimum turbidity in TCMPPJ group (i.e., 27.35 NTU) than all other groups during storage at 4 °C for three months. The decreasing turbidity values of the tested pomegranate juice samples under different clarification conditions may be due to the chemical nature of the clarifier materials used in this study. As a result of this nature, some complexes were formed, like protein–carbohydrate (TAŞTAN & BAYSAL 2015) and phenolic - clarification agents (DIBLAN & ÖZKAN 2021).

Clarification treatment positively affected the total polyphenolic content of the juices. These values were decreased significantly from (1335.15 and 1344.23) mg GAE/l into values ranging between 301.34 and 646.24 mg GAE/l in the end of their storage period at 4 °C (Figure 5).



See the meaning of each abbreviation in Table 1. The values are obtained as average of three determinations of juice samples. For each time of storage, data with different lowercase letters refer to significant differences at (p<0.05).

Figure 4. Effects of clarification treatment, pasteurization method and storage time on turbidity (NTU) of pomegranate juice.



See the meaning of each abbreviation in Table 1. The values are obtained as average of three determinations of juice samples. For each time of storage, data with different lowercase letters refer to significant differences at (p<0.05).

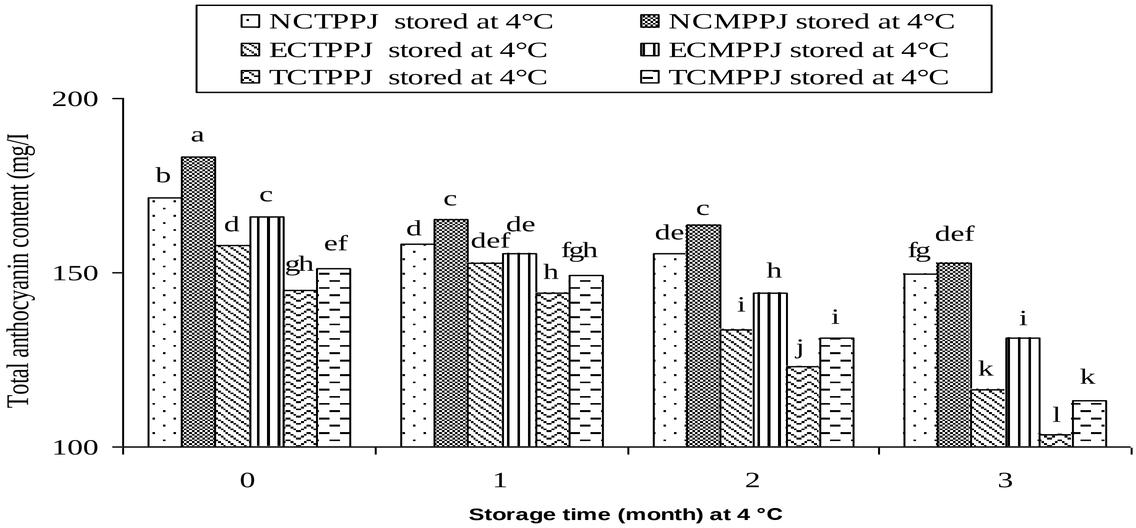
Figure 5. Effects of clarification treatment, pasteurization method and storage time on polyphenolic content (mg/l) of pomegranate juice.

When the total polyphenol contents were examined, the juice samples clarified by the enzymes contained the highest polyphenolic contents compared with those clarified using chitosan plus gelatin together (Figure 5). The results reported in Figure 5 showed that the pomegranate juices clarified with two techniques and pasteurized by using electromagnetic radiation had polyphenolic values of 646.24 and 339.03 mg/l greater than thermal water bath pasteurized samples with 585.06 and 301.34 mg/l, respectively, after the end of storage at 4 °C. The above results are in agreement with other papers (VARDIN & FENERCIOGLU 2003, ALPER et al. 2005, TURFAN et al. 2011).

Figure 6 shows that the results of the total anthocyanin contents were higher, significantly decreasing in the state of clarification with traditional clarifiers than enzymatic ones, compared with untreated clarifiers, as the values reduced into (15.47 and 17.60) %, for traditional clarification.

The results also showed that total anthocyanin contents were 171.48, 157.77, and 144.95 mg/l, in thermally pasteurized (NCTPPJ, ECTPPJ and TCTPPJ) juice samples, being less than that for the same juice samples pasteurized by microwave (183.38, 166.10, 151.10) mg/l at 0 month of storage, respectively.

There were significant differences (p<0.05) among anthocyanin values of the juices during the entire storage at 4 °C for three months. The reduction of the anthocyanin values could be attributed to the sensitivity of anthocyanins against clarifying agents. In general, the results of our research were consistent with the findings of other studies (CERRETI et al. 2017).



See the meaning of each abbreviation in Table 1. The values are obtained as average of three determinations of juice samples. For each time of storage, data with different lowercase letters refer to significant differences at (p<0.05).

Figure 6**.** Effects of clarification treatment, pasteurization method and storage time on total anthocyanin content (mg/L) of pomegranate juice.

The results of this study suggest an important implication of traditional clarification mixture composed of gelatin and chitosan in the reduction of turbidity and maintain the physicochemical properties in pomegranate juice during cold storage, furthermore, the enzymatic clarification mixture has potential implication in relation to pomegranate juice in terms of cloudiness and other characteristics are encouraged. The importance of obtained results for the market is addition more information about quality of clarified pomegranate juice during storage for several months. The effect of clarification, pasteurization, and storage conditions applied with this research are needed in the future studies to evaluate their effects on the other pomegranate juice characteristics, and also on quality of other cloudy fruit juices.

CONCLUSION

This study provides data on the effects of different treatments of cold stored pomegranate juice on its some quality properties. The results show that treatment (TCMPPJ) could be applicable for the production of clarified pomegranate juice and still maintain a good quality for the market.

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