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EVALUATION OF CHITOSAN AS A NEW NATURAL PRESERVATIVE IN ORANGE JUICE

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ABSTRACT

Orange juice is one of the most widely consumed fruit juices in the world, as it provides a nutritious option with a pleasant flavor for a wide variety of customers. As a result, it has the potential to deliver useful substances into the human body, such as vitamin C and phenols. The present study was designed to produce orange juice by incorporating chitosan at different proportions (0.08-0.12 g/100 ml juice) into freshly produced orange juice.

The sample formulations were further analyzed for changes in their physicochemical including, pH, titratable acidity, total soluble solids, the ratio of total soluble solids to acidity, turbidity, ascorbic acid content, and total phenol content. Microbiological and sensory properties including taste, color, odor, and overall acceptability were determined. As well as their storage stability over a 30-day storage period, the orange juice exhibited improved physicochemical and shelf life properties based on the respective chitosan concentrations used.

Hence, the study concluded that chitosan could be used as a natural preservative in the formulation of orange juice.

Keywords: Chitosan*;* Orange juice; Natural preservative; Storage; Sensory properties; TCA.

INTRODUCTION

Preservatives are chemical compounds applied to food to prevent or postpone microbial, oxidative, and enzymatic degradation. Using chemicals for preservation has several negative repercussions, such as sulfites, which are commonly used as preservatives in fruits and can cause headaches and cancer. As

a result, recent food preservation advancements have focused on the utilization of natural antioxidant and antibacterial compounds (Amit *et al.,* 2017).

Chitosan is a linear amino-polysaccharide derived from the alkaline deacetylation of chitin, which is considered to be the second most natural biopolymer in nature, after cellulose (Trache, 2018 & Trache *et al.,* 2020). Chitin is a polymer of (1 →4) N-acetyl-D-glucosamine units mainly found in fungi cell walls and the exoskeletons of insects and crustaceans, e.g., shellfish and crabs. Chitosan is a copolymer of glucosamine monomers and a few N-acetyl glucosamine monomers. The free amino groups turn chitosan into a cationic form. The positive ionic charge of chitosan allows it chemically binds to fats, bile acids and targeting the bacterial negative cytoplasmic membrane. Therefore, chitosan is antibacterial, antioxidant, antitumor, widely available, biocompatible, biodegradable, and void of toxicity (Sabu *et al.,* 2020). Moreover, chitosan film-forming and good gelling properties make it a versatile tool for a wide range of applications, including wastewater treatment (Sarode *et al.,* 2019), biotechnology, agriculture, aquaculture, textiles, food applications, environmental and pharmacology studies and applications (Chattopadhyay *et al.,* 2019).

Orange juice is one of the most widely sold fruit juices, and it is also one of the most well-known and accepted. Orange juice's great economic value stems from its pleasant sensory characteristics (i.e., odor, taste, and color), as well as its high vitamin C, natural antioxidant content, carotenoids, and phenolic compounds (Bull *et al.,* 2004; Rivas *et al.,* 2006 & Martí *et al.,* 2009).

The low shelf-life of orange juice is the most significant barrier to commercial marketing (Cortés *et al.,* 2006 & Cortés *et al.,* 2008). Three of the most common reasons for quality loss during the shelf-life of this product are microbial spoilage, off-flavor development, and ascorbic acid depreciation. Furthermore, the Food and Drug Administration has advised customers to avoid consuming orange juice products due to the risk of Salmonella typhimurium contamination and its link to a human disease epidemic caused by this organism (FDA, 2005).

So, the aim of this study was to employ chitosan as a natural preservation ingredient instead of harmful preservatives to develop a functional drink and evaluate the effect of chitosan on the physicochemical properties of orange juice during storage.

MATERIALS AND METHODS

1. Materials

1.1. Raw materials

Fully ripe orange fruits were purchased from a local market in Zagazig City, El- Sharkiya Governorate, Egypt. Commercial chitosan with deacetylation degree (DD 75%) was purchased from Loba Chemie Pvt. Ltd. for High-Grade Laboratory Reagents and Fine Chemicals Mumbai, India.

1.2. Reagents

All the chemicals and solvents utilized in this investigation were purchased at the highest analytical grade or extra purity. Hydrochloric acid (HCl) was purchased from SD Fine-Chem Limited, India. Sodium Hydroxide pellets (NaOH), Sodium carbonate (Na_2CO_3), Folin Ciocalteu reagent, and Sodium benzoate were purchased from Loba Chemie Pvt. Ltd. for High-Grade Laboratory Reagents and Fine Chemicals Mumbai, India.

2. Methods

2.1. Preparation of orange juice

The fruits were sorted, cleaned, and washed under running water. Using a sharp stainless-steel knife, the cleaned fruits were peeled to remove the pericarp and seeds. The orange fruit juice was extracted using the method reported by Akusu *et al*. (2016). To get clear juice, the fruit juice was manually removed and then filtered through a double-layered filtering mesh.

Commercial chitosan (75%) and sodium benzoate (chemical preservative) were added to the juices at values of 0.08 and 0.12 g/100 ml juice. The enriched juice was immediately packed in sanitized airtight plastic bottles and pasteurized at 80 °C for 10 minutes.

The orange juice was then quickly chilled in cold water for two minutes. The juices were kept at 4 °C (±1 °C) in the refrigerator. The samples were stored for one month during which further analyses were conducted on the first day of manufacturing, after 15 days, and after 30 days of manufacturing.

2.2. Physicochemical properties of orange juice

2.2.1. pH measurement

At room temperature and continuous stirring, the pH of 20 mL samples was measured using a pH-meter (HI 9811-5, USA) according to AOAC (2000) method.

2.2.2. Total soluble solids (T.S.S.) °(Brix)

Brix was calculated by measuring the index of refraction with a refractometer (Digital Hand-Held Refractometer with Automatic Temperature Compensation (ATC), USA) according to AOAC (2000) method.

2.2.3. Titratable acidity (TA)

A conical flask was filled with 10 ml of juice and 25 ml of distilled water. The mixture was titrated against 0.1N NaOH after three drops of

phenolphthalein (as an indicator) were added. Titration was continued until a pink coloration was noticed and a burette reading was taken. The sample was replaced with distilled water for the blank titration (AOAC, 2000). TA was calculated as in Eq. (1).

(1) $$TA\ \% = \left(\frac{\text{Molarlity of NaOH } \mathbf{X} \text{ (Titre of sample – blank) } \mathbf{X}\ 0.0640}{\text{Sample volume}}\right) \text{X100}$$

Where: 0.06404 = ml equivalent of citric acid.

2.2.4. *Turbidity*

The turbidity of each juice sample was determined using a turbidity meter (HI 98703, USA). A 1:25 (juice/water) solution was used to measure the orange juice samples turbidity.

2.2.5. *Determination of total phenolic content*

The Folin-Ciocalteau method (Nabavi *et al.,* 2008) was used to determine the total phenolic component contents. The samples (0.5 ml of various dilutions) were combined for 5 minutes with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and then added to aqueous Na_2CO_3 (4 ml, 1 M). The phenols were measured using colorimetry at 765 nm after the mixture had been left for 15 minutes. The standard curve was made out of gallic acid solutions in methanol: water (50:50, v/v) at concentrations of 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5 mg ml^{-1}. The concentration of total phenolic content (mg ml^{-1} juice) was calculated using the following straight-line equation with $R^2 = 0.9945$:

$$y = 3.8126x + 0.0379$$

Where: (y) the optical density, (x) the concentration in mg ml^{-1}

2.1.6. Ascorbic acid content

A 250 mL conical flask was filled with an aliquot of the orange juice samples (20 ml) solution, 2 ml of oxalic acid, roughly 150 ml of distilled water, and 1 ml of starch indicator solution. With a 0.005 mol L^{-1} iodine solution, the samples were titrated. The first distinct trace of a dark blue-black color caused by the development of the starch-iodine combination was identified as the titration's endpoint. Titrations with additional aliquots of sample solution were performed until concordant findings (titers within 0.1 ml) were achieved (Ismail *et al.*, 2014). Ascorbic acid content was calculated in Eq. (2):

(2) $$\text{Ascorbic acid content} = \left(\frac{\text{V X N X 100 X176.12}}{\text{volume of sample } x\ 2}\right)$$

Where: (V) volume of iodine, (N) normality of iodine, (176.12) molecular weight of ascorbic acid.

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2.1.7. Microbiological analyses of orange juice

Throughout the storage period, the samples were subjected to microbiology tests at regular intervals (the first day, 15 days, and 30 days of manufacturing). 1 ml of decimal dilution of samples was pipetted to Petri dishes. An enumeration of total counts was conducted at 30 °C on plate count agar (PCA) over 72 h. The results were expressed as log 10 colony forming units per ml (CFU ml^{-1}).

2.1.8. Sensory evaluation of orange juice

Sensory characteristics of the orange juice samples were evaluated based on color, taste, odor, and overall acceptability. Sensory attribution was carried out by 26 members aged 18-68 years. We examined orange juice sensory qualities using the general scoring technique, which was derived by multiplying the supplied values by sensory indicators ranging from 1-4. (1 = unacceptable, 2 = acceptable, 3 = good, and 4 = excellent).

2.1.9. Statistical analysis

Experiments were carried out in triplicate, and data were evaluated using analysis of variance (ANOVA). The Tukey's multiple range tests (SPSS version 20) was used to separate the means, and the findings were considered significant at the 5% level (p 0.05).

RESULTS AND DISCUSSION

1. *Physicochemical properties of orange juice*

1.1. Changes in pH of orange juice during storage

pH has long been an essential parameter in determining the quality of food items, notably fruit juices. Chitosan concentration and storage duration had a significant impact on pH compared to control and sodium benzoate samples. Figure (1) shows the changes in pH of orange juice samples as affected by storage. On the first day of manufacturing, pH increased significantly with increasing chitosan concentration compared to control and sodium benzoate samples. These results agree with Martín-Diana *et al.* (2009). This effect might be attributed to chitosan's having a positive charge

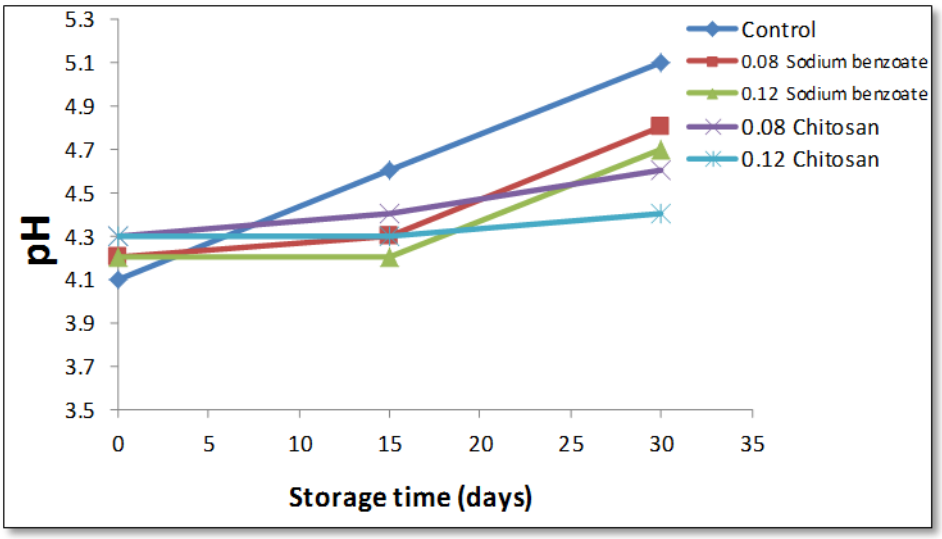

Fig. 1. Changes in pH of orange juice during 30 days of storage under refrigeration conditions with sodium benzoate and chitosan as preservatives at a concentration of 0.08 and 0.12 g/100 ml juice.

along its backbone when the pH is less than 6.5, which leads to chitosan's ability to decrease fruit juice acidity due to its acid-binding ability (Einbu & Varum, 2003). The pH values increased with the increasing storage period. According to the findings of this study, the control sample and sodium benzoate samples exhibited a continuous great increase in pH values from storage day 0 to day 30. The samples containing different chitosan concentrations showed a very slight (2009); Ghasemnezhad *et al.* (2010); Domingues *et al.* (2012).

1.2. Changes in T.S.S. of orange juice during storage

Figure (2) depicts the total soluble solids of orange juice samples. T.S.S. is measured of total dissolved solids, which are often sugars and acids found in food, using the mechanism of light's refractive index while passing through different mediums (Osungbade *et al.*, 2021). Chitosan reduced the Brix value of the orange juices. This may be related to the ability of the positively charged polysaccharide to bind to negatively charged components, which works on coagulate suspended solids (Sapers, 1992). Sodium benzoate at 0.08 and 0.12 g/100 ml showed the highest T.S.S. values on the first day of manufacturing. It may have led to an increase in the concentration of T.S.S. in the sample. The T.S.S. levels of the control sample decreased consistently with storage time,

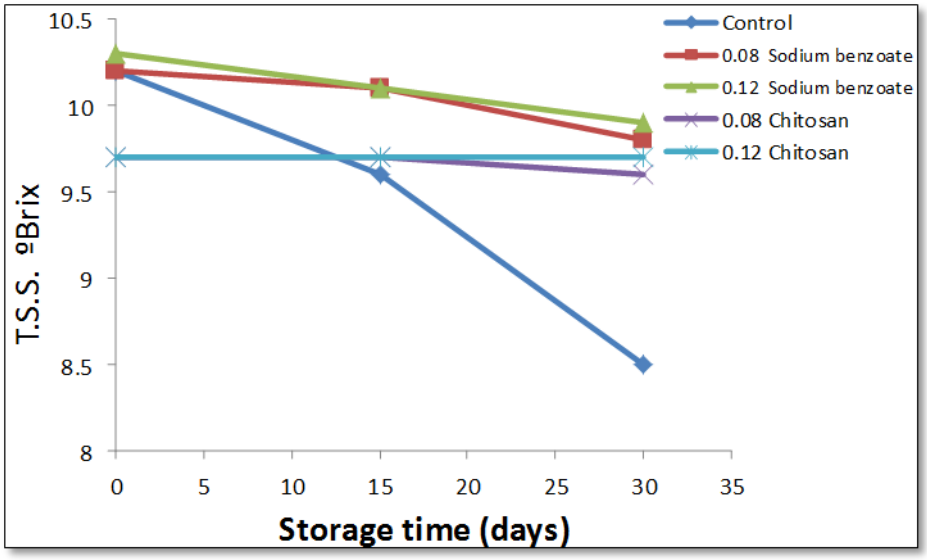

Fig.2. Changes in total soluble solids of orange juice during 30 days of storage under refrigeration conditions with sodium benzoate and chitosan as preservatives at a concentration of 0.08 and 0.12 g/100 ml juice.

while sodium benzoate samples exhibited slightly decreased T.S.S. values during storage periods. This may be due to the degradation of solids and sugars resulting from increased bacterial activity throughout the storage period. There were no notable alterations noticed in chitosan samples during the period of storage in T.S.S. values as shown in Figure (2). These results were in agreement with Ayhan *et al.* (2001); Bull *et al.* (2004); Rivas *et al.* (2006); Cortés *et al.* (2008).

1.3. Changes in titratable acidity (TA) of orange juice during storage

Titratable acidity (TA) is often used to indicate the organic acid component of juice. These organic acids are extremely nutritious (Singh and Sharma, 2017). The titratable acidity of orange juice samples was altered throughout a 30-day storage period, as shown in Figure (3). There was found an inverse connection between the pH values obtained, i.e. the titratable acidity values dropped with storage time, in agreement with Kale *et al.* (2012). In this study, the control sample had the lowest values throughout the storage period. TA values for sodium benzoate samples decreased during storage periods, whereas there were no significant changes seen in chitosan samples over the storage period. These findings are consistent with Ghasemnezhad *et al.* (2010); Belgheisi & Esmaeil Zadeh (2019).

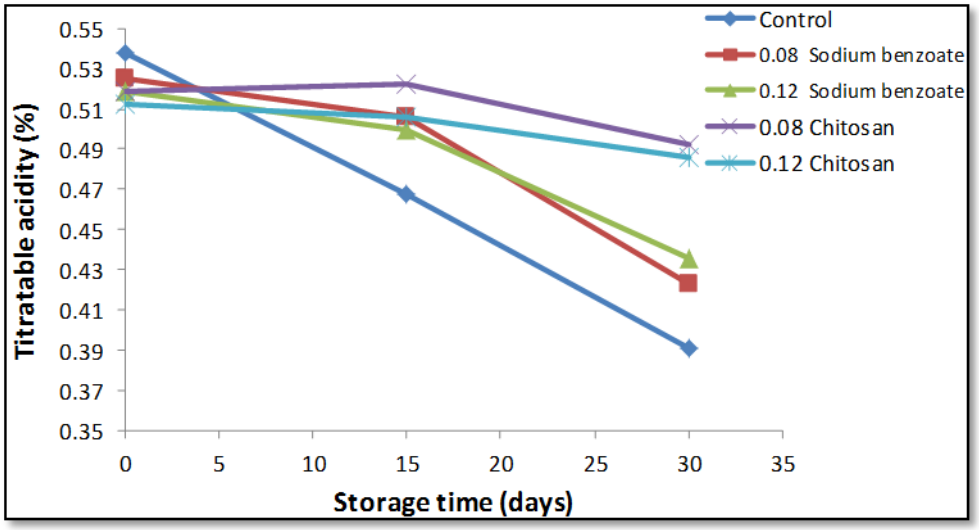

Fig.3. Changes in titratable acidity (%) of orange juice during 30 days of storage under refrigeration conditions with sodium benzoate and chitosan as preservatives at a concentration of 0.08 and 0.12 g/100 ml juice.

The reduction in TA over time might be due to the gradual oxidation of ascorbic acid and conversion of acid to sugar during storage (Bhardwaj and Nandal, 2014). This confirms that chitosan reduces oxidative processes in food systems during storage periods.

1.4. Changes in ratio of T.S.S. °(Brix) to titratable acidity (%) of orange juice during storage:

Brix value, acidity, and the Brix-acidity ratio are standard quality indicators used to assess the sweetness, maturity of the fruits, and tartness from which the juice was collected (Utama, 2015). In this study, the T.S.S.: TA ratio was increased in the control sample and sodium benzoate samples, which might be related to the faster rate of acidity reduction during storage. There was a slight increase in T.S.S.: TA ratio in chitosan samples over the storage period, as shown in Figure (4), and these findings are consistent with Haider *et al.* (2017).

1.5. Changes in turbidity (NTU) of orange juice during storage

Figure (5) depicts the change in turbidity of the control sample and at various chitosan and sodium benzoate concentrations during storage times. Turbidity decreases in chitosan samples when varied concentrations of chitosan are added. These findings were consistent with Chatterjee *et al.* (2004) observed a reduction in turbidity as a result of chitosan addition. Rao *et al.*

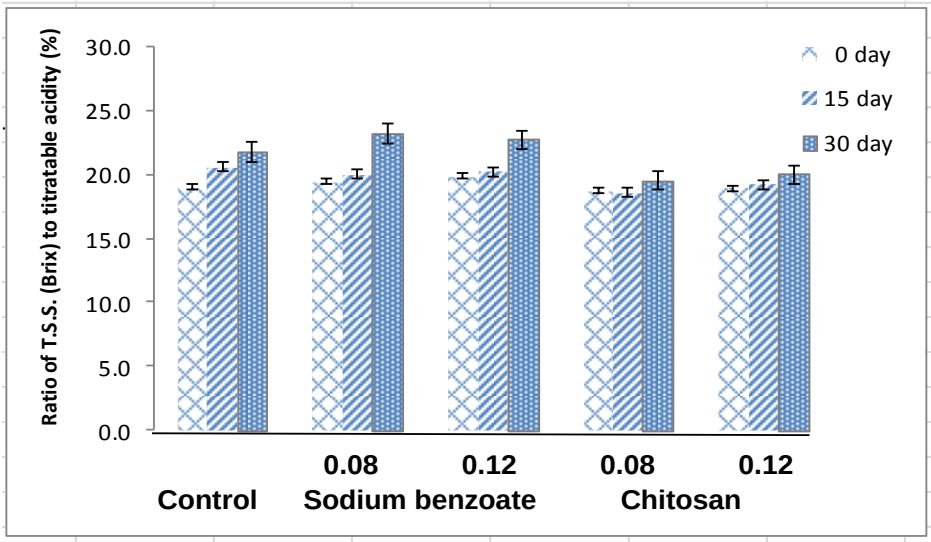

Fig.4. Changes in total soluble solids to titratable acidity (%) of orange juice during 30 days of storage under refrigeration conditions with sodium benzoate and chitosan as preservatives at a concentration of 0.08 and 0.12 g/100 ml juice.

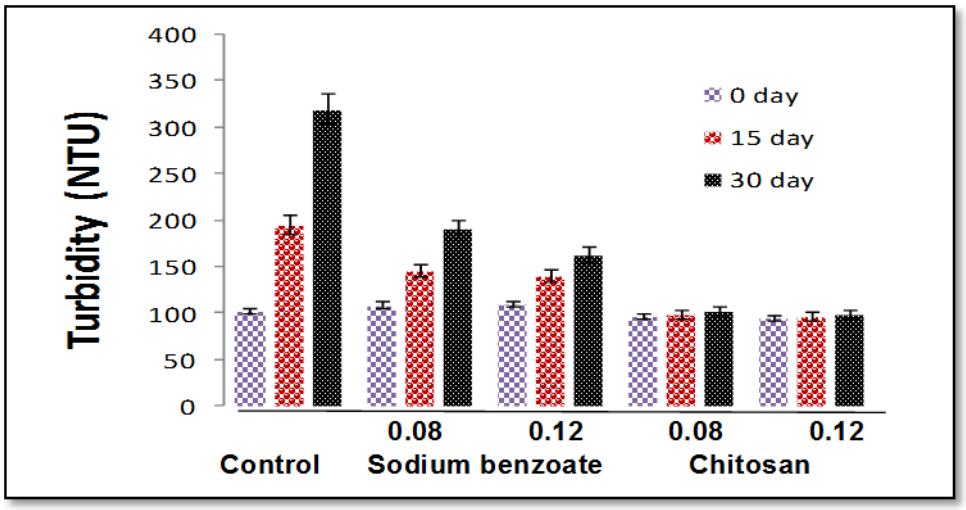

Fig.5. Changes in turbidity (NTU) of orange juice during 30 days of storage under refrigeration conditions with sodium benzoate and chitosan as preservatives at a concentration of 0.08 and 0.12 g/100 ml juice.

(2011) also found that chitosan may coagulate anionic components such as pectin and protein and subsequently separate the suspended particles from the beverage, reducing its turbidity. This activity is connected to chitosan physicochemical characteristics, which are related to the presence of amine group functionalities (Marudova *et al.,* 2004). In the current investigation, there was a significant rise in turbidity in control and sodium benzoate samples throughout storage periods, but only a very minor increase in turbidity in chitosan samples during storage periods. These results are in agreement with Martín-Diana *et al.* (2009) who reported that the turbidity of the orange juice was controlled by the concentration of chitosan.

1.6. Changes in total phenolic contents (TPC) of orange juice during storage

Figure (6) depicts the trend in TPC of orange juice samples throughout a 30-day storage period. The phenolic compounds most commonly found in food items include flavonoids, anthocyanins, catechins, and others, and they are significant because they have antioxidant capabilities. Hence, a link appears to exist between the TPC and the antioxidant qualities of food products (Osungbade *et al.,* 2021). In the current investigation, the phenolic compounds in chitosan samples were found to be greater than in control samples as indicated in the figure (6). The outcome contradicts the findings of Liu *et al.* (2007). The control sample showed the fastest rate of decline on TPC during the 30 days of storage. During the 30 days of storage of the chitosan samples, there is a very minor decrease in TPC. These results agree with Ghasemnezhad *et al.* (2010). The reduction in TPC during storage might be attributed to phenolic compounds' susceptibility to light and oxidation (Ali *et al.,* 2018).

1.7. Changes in ascorbic acid content of orange juice during storage

Vitamin C has been shown to play an important impact on both the nutritional and antioxidant properties of orange juice. Because the body is unable to synthesize this vitamin, we must consume it as part of our diet (Osungbade *et al.,* 2021). Ascorbic acid content has been cited as an indication of quality in juices. Orange juice is considered one of the greatest sources of vitamin C by consumers (Shaw & Moshonas, 1991). In the current investigation, the addition of greater chitosan concentrations resulted in a reduction in ascorbic acid content compared to the control sample, consistent with Martín-Diana *et al.* (2009). After 15 and 30 days of storage, there are no changes in the ascorbic acid contents of any of the chitosan samples, as shown in Figure (7). But, during the storage periods, the control and sodium benzoate samples exhibited a rapid rate of decrease.

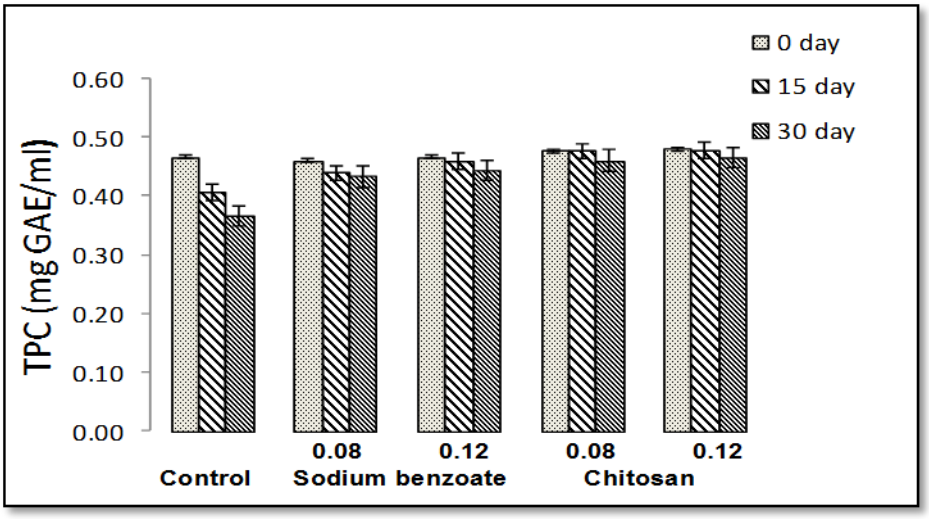

Fig.6. Changes in total phenolic contents of orange juice during 30 days of storage under refrigeration conditions with sodium benzoate and chitosan as preservatives at a concentration of 0.08 and 0.12 g/100 ml juice.

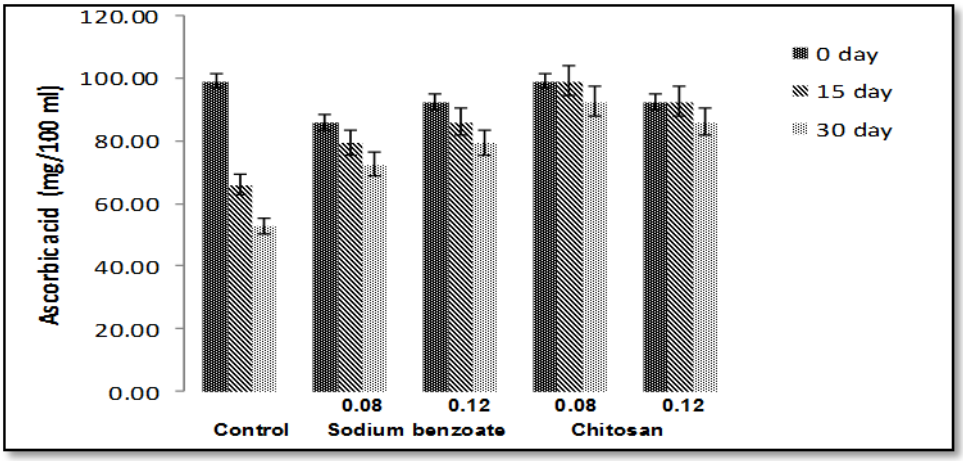

Fig.7. Changes in ascorbic acid content of orange juice during 30 days of storage under refrigeration conditions with sodium benzoate and chitosan as preservatives at a concentration of 0.08 and 0.12 g/100 ml juice.

1.8. Microbiological analyses of orange juice

The results of a Total Aerobic Count (TAC) test are an indication of bacterial contamination in orange juice. In the food industry, testing labs use TAC tests to gauge the sanitary quality of the food systems. Figure (8) displays

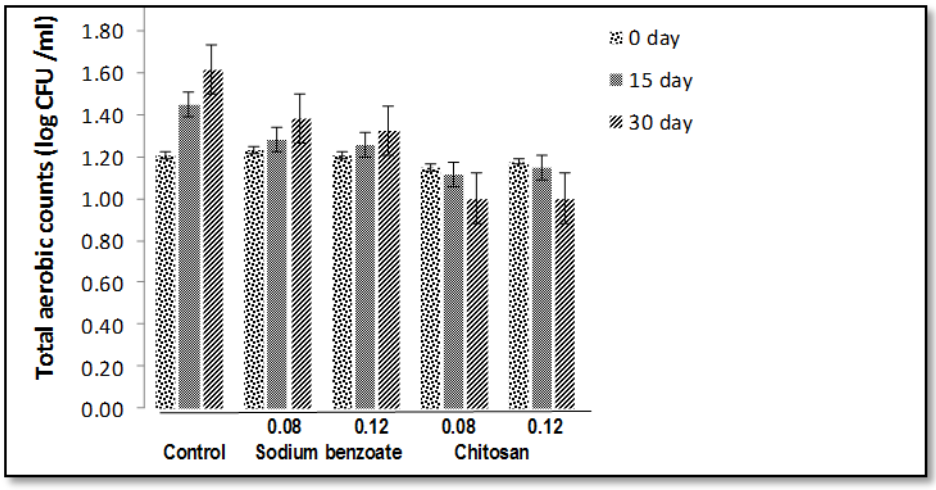

Fig.8. Changes in total aerobic count (TAC) of orange juice during 30 days of storage under refrigeration conditions with sodium benzoate and chitosan as preservatives at a concentration of 0.08 and 0.12 g/100 ml juice.

the TAC during storage in orange juice. The TAC decreased in orange juice samples after the addition of different concentrations of chitosan. The TAC greatly increased in the control sample of orange juice during the 30 days of storage. TAC was significantly reduced in orange juice samples containing different concentrations of chitosan after 15 and 30 days of storage. These findings agree with Martín-Diana *et al.* (2009) who found that chitosan concentration considerably reduced the TAC of orange juice, resulting in a beneficial effect on shelf-life extension. These decreases in aerobic counts might be linked to a chitosan activity for antibacterials that develops with storage time (Kisko *et al.*, 2005 & No *et al.*, 2007).

1.9. Evaluation of sensory characteristics of orange juice

The effect of chitosan on consumers' preferences for orange juice in terms of taste, color, odor, and overall acceptability is presented in Figures (9, 10, 11 and 12, respectively). Based on the evaluation, most of the judges perceived no difference between orange juice samples that contained chitosan during the storage period. The color, odor and general acceptability evaluation of orange juice samples implies that the chitosan treatments had no significant effect on the color, odor and general acceptability. The taste evaluation analysis of orange juice samples that contained chitosan showed that all these samples had

no significant effect on taste during the storage period from the first day to 15 days. A very slight reduction in taste was observed in chitosan samples after 30

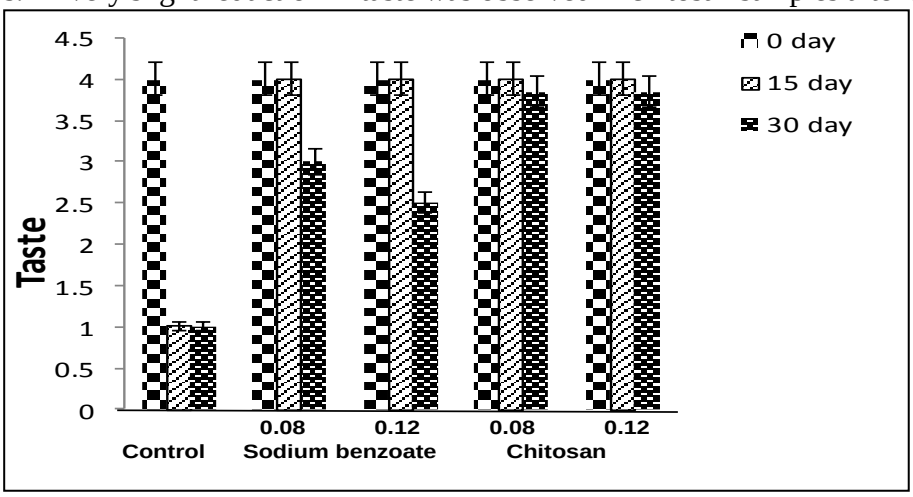

Fig.9. Changes in taste of orange juice during 30 days of storage under refrigeration conditions with sodium benzoate and chitosan as preservatives at a concentration of 0.08 and 0.12 g/100 ml juice.

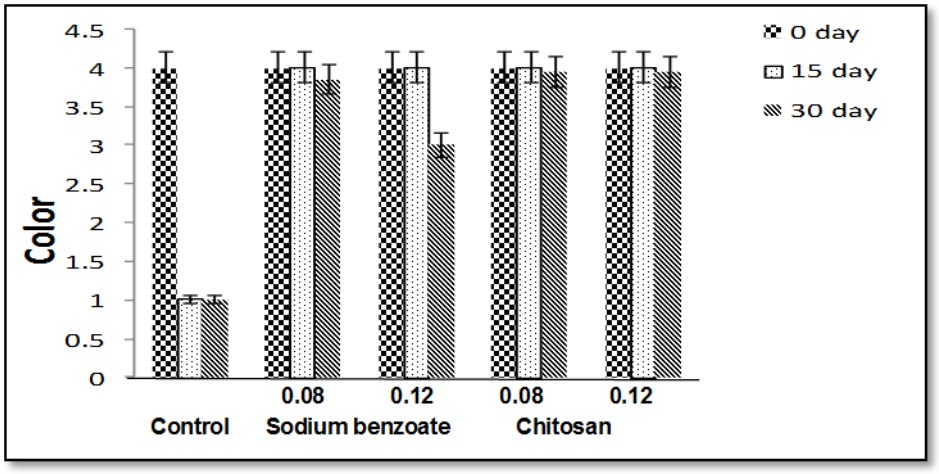

Fig.10. Changes in color of orange juice during 30 days of storage under refrigeration conditions with sodium benzoate and chitosan as preservatives at a concentration of 0.08 and 0.12 g/100 ml juice.

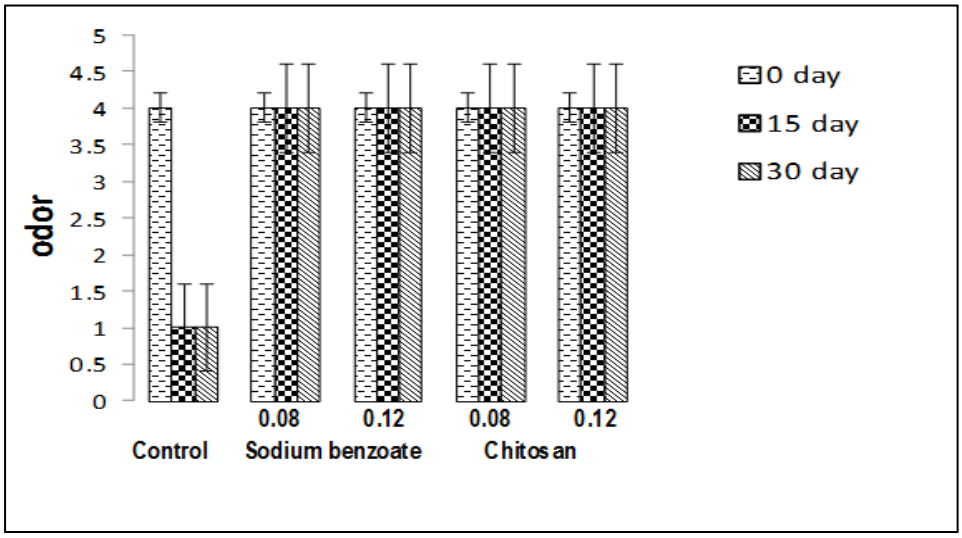

Fig.11. Changes in odor of orange juice during 30 days of storage under refrigeration conditions with sodium benzoate and chitosan as preservatives at a concentration of 0.08 and 0.12 g/100 ml juice.

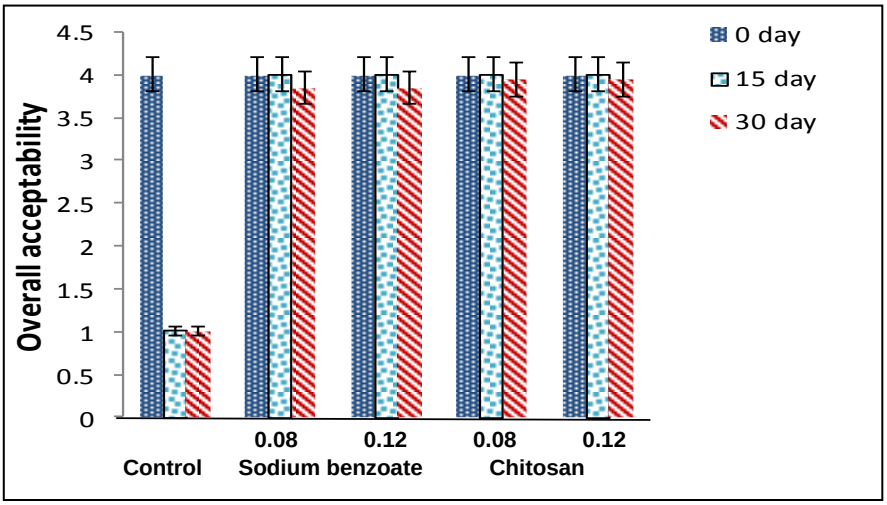

Fig.12. Changes in overall acceptability of orange juice during 30 days of storage under refrigeration conditions with sodium benzoate and chitosan as preservatives at a concentration of 0.08 and 0.12 g/100 ml juice.

days of storage. These findings are in agreement with Martín-Diana *et al.* (2009) who observed that juices enhanced with chitosan were deemed

acceptable at the end of storage, even though the starting values were somewhat lower than those of fresh orange juice. Chitosan can reduce the acidity of orange juices. This effect was reflected in the improvement in the organoleptic properties, consistent with Han *et al.* (2005).

Conclusively, this study found that incorporating chitosan at concentrations of 0.08-0.12 g/100 ml into orange juice manufacturing improved the overall physicochemical, microbiological, and sensory properties. Over 30 days, the shelf-life stability of orange juice was improved in terms of these characteristics. As a result, chitosan is a promising food preservation material.

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تقييم الكيتوزان كمادة حافظة طبيعية جديدة في عصير البرتقال

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يعد عصير البرتقال من أكثر أنواع عصائر الفاكهة استهلاكًا في العالم ، فهو من أكثر العصائر المغذية ذات النكهة لدى قطاع كبير من المستهلكين. نتيجة لذلك ، كما إنه يمد الجسم بالمواد المفيدة، مثل فيتامين ج والفينولات. صممت الدراسة الحالية لإنتاج عصير برتقال بخلط الكيتوزان بنسب مختلفة (0,08-0,12 جم / 100 مل عصير برتقال طازج). تم تحليل العينات لمعرفة التغيرات في الكيميائية الطبيعية مثل، الرقم الهيدروجيني، الحموضة القابلة، المواد الصلبة الذائبة الكلية، نسبة المواد الصلبة الذائبة الكلية إلى الحموضة، التعكر، محتوى حامض الأسكوربيك، ومحتوى الفينولات الكلية. تم تقدير الخصائص الميكروبيولوجية والحسية مثل, الطعم واللون والرائحة والقبول العام. بالإضافة إلى استقرار تخزينها، نتج عن إضافة الكيتوزان بتركيزات مختلفة تحسن خصائص العصير الكيميائية الطبيعية وزيادة مدة صلاحيتة.

التوصية: خلصت الدراسة إلى أنه يمكن استخدام الكيتوزان كمادة حافظة طبيعية كإضافة لعصير البرتقال.