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# STUDY ON THE STORAGE STABILITY OF CHIA SEEDS INCORPORATED POMEGRANATE SYRUP AND RTS

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**ABSTRACT**

Enhancing product quality has become essential in contemporary fruit beverage processing, contributing to the creation of trendy beverages with exceptional taste and beneficial health properties. The captivating appearance, enticing flavour, nutrient retention, and enrichment are key considerations that attract consumers preferences and enhance the marketability of new products. In addition to the clear benefits of pomegranate fruit and chia seeds, the creation of blended pomegranate syrup with chia seeds is an exciting value addition. The study aimed to evaluate the biochemical parameters and sensory assessment prepared syrup after three months storage period. The syrup containing chia seeds incorporated pomegranate stored for three months in glass bottles at refrigerated (4ºC) temperature were evaluated at 9-point hedonic scale for their colour, flavour, consistency, taste and overall acceptability. Where all the recipes were liked moderately to very much. Organoleptic evaluation of syrup revealed a decreasing trend with the advancement of storage period. In case of syrup based RTS, maximum sensory score for colour and appearance was recorded in $T_1$ (7.88). The highest sensory score for overall acceptability was noticed in $T_6$ (7.01). The highest antioxidant activity (57.28%) and total phenol content (229.73) were recorded in $T_7$. In case of syrup, the highest antioxidant activity (100.42%) and total phenol content (629.55) was recorded in $T_7$. There was decline in the sensory quality of syrup with prolonged storage period. Therefore, it can be clarified that creating a beverage combining pomegranate juice and chia seeds is highly suitable for satisfying consumer preferences and tastes.

**Keywords:** pomegranate, chia seeds, syrup, antioxidant, phenols, sensory evaluation

## Introduction

Storage stability is the ability of the product to maintain its quality, effectiveness and safety characteristics over a specific period of time under defined storage conditions. It is an essential requirement for product to maintain its quality, efficacy and safety of product throughout the intended shelf life and use.

*Punica granatum* L. (Pomegranate) is an imperative and highly gratitude fruit crop of tropical and subtropical zone. It is a member of '*Lythraceae*' family; it is identified as 'Fruit of Paradise'. Pomegranate word is acquired out of pomum (apple) and granates (seeded). Regionally it is familiar as *Dhalimbe*. Balusta is the morphological term used to describe it. It is extensively cultivated in Iran, Spain, Turkey, Egypt, Russia, Japan, USA and India. In the world, India is the number-one ruling country in the production of pomegranate. The estimated global cultivated sector under pomegranate is about 3 lakh hectares and production is 3.0 million tonnes. In India, pomegranate fruits are extensively grown in different states like Maharashtra, Gujarat, Karnataka, Andhra Pradesh and it is accelerating in Madhya Pradesh, Rajasthan and Himachal Pradesh. As per the latest progress evaluation for the year 2021-22, total area under pomegranate in India is 246 thousand hectares among 78,000 hectares is in Maharashtra at best and entire production in India is 3212.33 thousand Tonnes (Anon., 2024). Pomegranate has latterly been admired for its health comfort, especially for its disease fighting antioxidant potential *viz.*, ellagic acid, punicalagin and gallic acid. These are rich in antioxidants which can encourage health by destructing free radicals (Rosenblat *et al*., 2006). The antioxidant activity of pomegranate cultivars was found between 15.59 and 40.7 per cent Tehranifar *et al.,* (2010). Cam *et al*., (2009) reported that the total phenolic content (TPC) and total anthocyanin content (TAC) vary between the range of 208.3–343.6 mg catechin equivalents and 8.1–36.9 mg cyanidine-3-glucoside equivalents per 100 ml of Pomegranate juice, respectively. Pomegranate seeds are used as a acidulant and condiments in Indian curries known as *anar dana* most notably in Indian and Pakistani cuisine. The dried whole seeds of pomegranate fruits are oftenly available in the ethnic markets. In Turkey, pomegranate sauce used to soak meat, simply to consume as refreshment or nourishment and as a salad dressing.

Chia seeds, scientifically known as *Salvia hispanica* L., are classified as an annual herb and are part of the Lamiaceae or Labiatae family. Chia seeds are tiny, oval-shaped seeds, which is native to Mexico. Salvia is derived from the word "Salvare", which translates to "The Healer" (Coorey *et al*., 2012). Chia seeds are rich in essential nutrients and are considered a superfood. They are a great source of omega-3 fatty acids (AHA - Alpha linolenic acid, EPA - eicosapentaenoic acid and DHA - docosahexaenoic acid), fiber, amino acids, phytonutrients (free radical scavenging activity) and micronutrients consisting of calcium, magnesium and phosphorus. Outlandish properties of seeds is the ability to imbibe the liquid and resulting a gel-like substance when mixed with water or other liquids, this consistency help with hydration and can be used as a substitute for eggs in baking recipes or as a thickening agent in various dishes. They can be sprinkled on top of cereals, yogurt, or salads, added to smoothies or baked goods, or used as a thickening agent in puddings and sauces. Although chia seeds have many health advantages, it's important to remember that they should only be eaten occasionally as part of a balanced diet (Segura-Campos *et al*., 2014). The nutritional properties of the main protein fractions and a protein isolate of chia seed was studied and found that it contain high amounts of glutamic acid (123.00 g $kg^{-1}$ raw protein), arginine (80.60 g $kg^{-1}$ raw protein) and aspartic acid (61.30 g $kg^{-1}$ raw protein) as reported by Olivos-Lugo *et al.,* (2010). Protein isolate showed a good water holding capacity (4.06 $100g^{-1}$) and an excellent oil holding capacity (4.04 $100g^{-1}$) as reported by Olivos-Lugo *et al.,* (2010).

Chia seeds are rich in dietary fiber and possess the unique ability to mop up water, expanding to form a gel-like mucilage substance in the stomach. This property makes chia seeds a beneficial option for individuals seeking low-carbohydrate, stomach-friendly foods (Alfredo *et al*., 2009). Chia superfood oftenly incorporated into various dishes and recipes, they can be added to smoothies, oatmeal, yogurt, salads, or used as an egg substitute in baking (Martnez-Cruz *et al*., 2014). Moreover, chia seeds can be wrapped in water to create a refreshing drink known as chia fresca, which can be enjoyed either on its own or mixed with fruit juice. Another option is mixing chia seeds with milk to enhance its nutritional value and create a nutrient-rich beverage (Coorey *et al*., 2014).

As nowadays consumers are increasingly seeking healthier and more functional beverage options, leading to the development of number of beverages with added nutrients. Despite the evident advantages of pomegranate fruit and chia seeds, the endeavor to explore the formulation of pomegranate syrup with added chia seeds poses a challenge. The goal is to assess the biochemical parameters and sensory attributes throughout the storage period.

## Materials and Methods

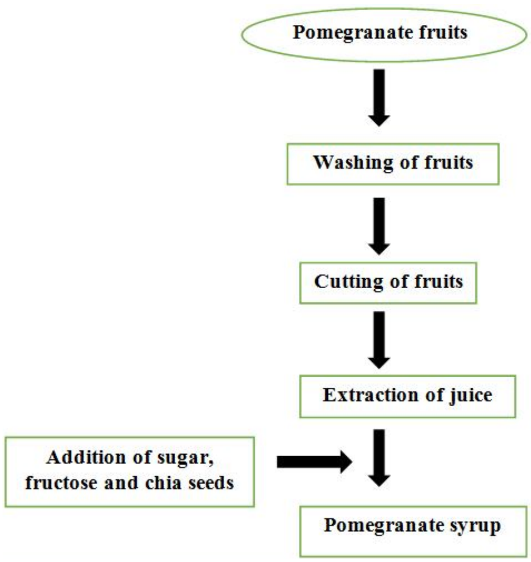
### Raw Materials

The pomegranate fruits for the experiment were acquired from the orchard of Mr. Younus located at Kaladagi, Bagalkot district. Chia seeds were bought from the Department of Vegetable Science, College of Horticulture, Bagalkot. Sugar and fructose used in this experiment was obtained from the local market, Bagalkot and Amazon shopping respectively.

## Mathodology

In the process of creating pomegranate juice, the cleanliness is ensured to eliminate potential diseases by thoroughly washing fresh pomegranate fruits under running water. Next, the fruits are halved using a sharp stainless-steel knife and the halved fruits are carefully placed in a hand-operated pomegranate juice extractor.

Through gentle pressing, the juice is extracted, preserving the integrity and flavours. Following the extraction, a settling period allows any sediment or impurities to separate, resulting in a clearer juice. Finally, the juice is strained through a clean muslin cloth to remove any remaining pulp, seeds, or solids, yielding a pure and clear juice extract.




The extracted pomegranate juice was divided according to the recipes, with fructose and sucrose utilized to maintain total soluble solids (TSS). Chia seeds were incorporated into all treatments as necessary, each with varying percentages (except $T_1$ and $T_5$) as per the treatment details given below. The processed syrup was poured into clean, sterile bottles, sealed tightly with caps and pasteurized at 85° C for 25 min. and stored in a refrigerator (4 °C). During the preparation of blended syrup sodium benzoate was added as a preservative at the concentration of 600 ppm.

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**Recipe details**

| Recipes | T1 | T2 | T3 | T4 | Recipes | T5 | T6 | T7 | T8 |
|---|---|---|---|---|---|---|---|---|---|
| PJ % | 100 | 90 | 85 | 80 | PJ % | 100 | 90 | 85 | 80 |
| CS % | - | 10 | 15 | 20 | CS % | - | 10 | 15 | 20 |
| Sucrose ° Brix | 65 | 65 | 65 | 65 | Fructose ° Brix | 45 | 45 | 45 | 45 |

**PJ**- Pomegranate juice; **CS**- Chia seeds

### Sensory Evaluation

The developed pomegranate syrup was diluted with water (1:3) to make into RTS. A semi-trained panel of 10 judges conducted a sensory evaluation of a syrup-based ready-to-serve (RTS) beverage. The sensory attributes assessed included appearance, colour, flavour, taste, palate and overall-acceptability. The sensory attributes were evaluated on the 9-point Hedonic scale, ranging from 1 (extremely dislike) to 9 (extremely like) by jury.

### Statistical Analysis

The quantifiable physico-chemical parameters and sensorial characteristics obtained during the research were analysed using a CRD (Completely Randomized Design). The interpretation of the results followed the guidelines (Panse and Sukhatme, 1985). A significance level of P = 0.01 was applied in the 'T' test to determine statistical significance. Whenever the 'T' test resulted in significance, critical difference values were computed. This rigorous statistical analysis helps ensure reliable and meaningful interpretation of the experimental results.

## Results and Discussion

### Sensory evaluation

The sensorial assessment of pomegranate ready-to-serve beverages enhanced with chia seeds is represented in Fig. 1 and Table 1. Among all the different recipes, 100 percent pomegranate juice recipe Control II $T_5$ (100 % PJ + Fructose 45° Brix ) obtained maximum score for colour and appearance (8.37) followed by recipe Control I $T_{1-}$ (100 % PJ + Sugar 65 °Brix) and recipe $T_4$ (80 % PJ + Sugar 65 °Brix + 20 % CS) scored least for colour and appearance. The evenness of the syrup was fair in the recipe $T_6$ (90 % PJ + Fructose 45 °Brix + 10 % CS : 8.00) and it was similar with recipe $T_1$ (100 % PJ + Sugar

**Table 1:** Sensory evaluation of pomegranate ready to serve beverage enriched with chia seeds.

| Recipes | C & A | C | T | F | OA |
|---|---|---|---|---|---|
| **$T_1$:** 100 % Pomegranate juice + Sugar (65 º Brix Control I) | 8.13 | 7.90 | 7.60 | 7.80 | 7.67 |
| **$T_2$:** 90 % Pomegranate juice + Sugar (65 º Brix) + 10 % chia seeds | 7.23 | 7.50 | 7.50 | 7.47 | 7.50 |
| **$T_3$:** 85% Pomegranate juice + Sugar (65 º Brix) + 15 % chia seeds | 6.70 | 7.37 | 7.37 | 7.27 | 7.10 |
| **$T_4$:** 80% Pomegranate juice + Sugar (65 º Brix) + 20 % chia seeds | 6.47 | 7.03 | 6.93 | 7.40 | 7.07 |
| **$T_5$:** 100 % Pomegranate juice + Fructose (45 º Brix Control II) | 8.37 | 7.70 | 7.67 | 7.63 | 7.77 |
| **$T_6$:** 90 % Pomegranate juice + Fructose (45 º Brix) + 10 % chia seeds | 8.03 | 8.00 | 7.73 | 7.93 | 7.87 |
| **$T_7$:** 85% Pomegranate juice + Fructose (45 º Brix) + 15 % chia seeds | 7.47 | 7.53 | 7.80 | 7.73 | 7.67 |
| **$T_8$:** 80% Pomegranate juice + Fructose (45 º Brix) + 20 % chia seeds | 7.00 | 7.47 | 6.97 | 7.17 | 7.23 |
| **Mean** | **7.42** | **7.56** | **7.45** | **7.57** | **7.48** |
| **S. Em±** | **0.16** | **0.06** | **0.14** | **0.12** | **0.13** |
| **CD @ 1%** | **0.65** | **0.33** | **0.54** | **0.48** | **0.57** |

**C & A:** Colour and appearance; **C:** Consistency; **T:** Taste; **F:** Flavour; **OA:** Overall acceptability

65 ºBrix : 7.90) and recipe $T_5$ (100 % PJ + Fructose 45 ºBrix: 7.70). Lowest score (7.03) for consistency was recorded in recipe $T_4$ (80 % PJ + Sugar 65º Brix + 20 % CS). The highest organoleptic score for taste (7.80) was in recipe $T_7$ (85 % PJ + Fructose 45 ºBrix + 15 % CS), flavour (7.93) and overall acceptability (7.87) was maximum in recipe $T_6$ (90 % PJ + Fructose 45 ºBrix + 10 % CS).

## Biochemical parameters

pH of syrup and syrup based RTS presented in Fig. 2 and Fig. 3. The pH scale is utilize to measure the acidity or alkalinity of a water based mixture. It quantifies the concentration of H+ ions in the solution. Measuring and understanding pH is important in many fields, including chemistry, biology, medicine, environmental science and agriculture. pH testing can be done using pH meters, pH indicator papers or pH probes.

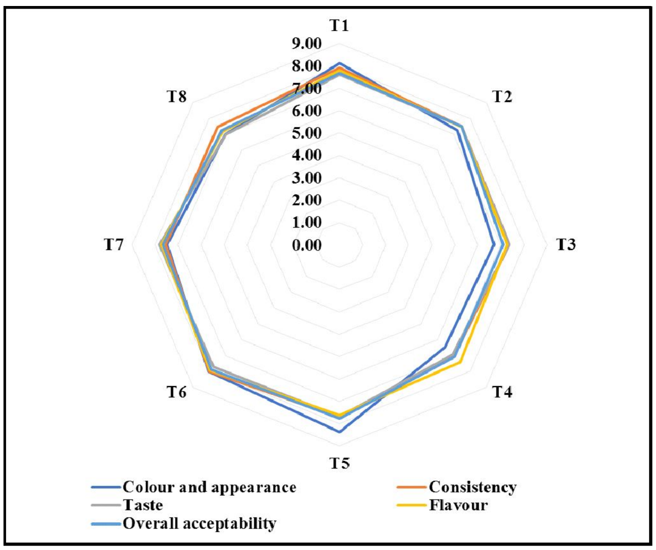


**Fig. 1:** Sensory evaluation of pomegranate ready-to-serve beverages incorporated with superfood seeds of chia. [**$T_1$:** 100 % Pomegranate juice + Sugar (65 º Brix Control I); **$T_2$:** 90 % Pomegranate juice + Sugar (65 º Brix) + 10 % chia seeds; **$T_3$:** 85% Pomegranate juice + Sugar (65 º Brix) + 15 % chia seeds; **$T_4$:** 80% Pomegranate juice + Sugar (65 º Brix) + 20 % chia seeds; **$T_5$:** 100 % Pomegranate juice + Fructose (45 º Brix Control II); **$T_6$:** 90 % Pomegranate juice + Fructose (45 º Brix) + 10 % chia seeds; **$T_7$:** 85% Pomegranate juice + Fructose (45 º Brix) + 15 % chia seeds; **$T_8$:** 80% Pomegranate juice + Fructose (45 º Brix) + 20 % chia seeds].

In case of syrup, significantly the highest value (3.43) noticed in recipe $T_4$ (80% PJ + Sugar 65 ºBrix + 20 % CS). The minimum mean value (2.57) for pH was noticed in recipe control II $T_5$ (100 % PJ + Fructose 45 ºBrix). In case of RTS the recipe $T_4$ (80 % PJ + Sugar 65 ºBrix + 20 % CS) possessed highest value (4.27) for pH and the lowest pH value was recorded in recipe control II $T_5$ (100 % PJ + Fructose 45º Brix : 2.84). With an increase in the percentage of chia seeds in the recipe, there was a corresponding rise in pH values observed in both the syrup and ready-to-serve (RTS) formulations. Similar results were reported in utilization gel of chia seed in ice cream (Chavan *et al*., 2017) and in low fat yoghurt (Darwish *et al*., 2018). The relationship between pH and viscosity depends on the attentiveness of mucilage. As the viscosity of the mucilage increases, pH levels also tend to rise as

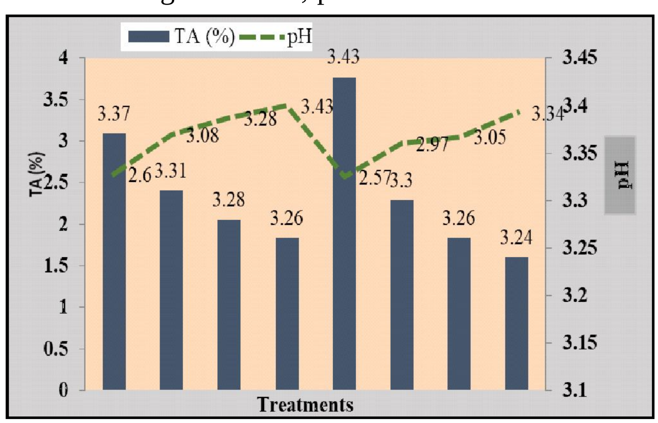


**Fig. 2:** pH and titratable acidity of chia based pomegranate syrup.

noted (Abdullah *et al.*, 2017; Capitani *et al.*, 2015 and Nduko *et al.*, 2018) in chia fortified pineapple jam. The lowest pH was noticed in the recipe T-$_5$ having 100 per cent pomegranate was reported (Amin *et al.*, 2018) in blended pomegranate and grapes RTS. Low pH of the fruit is ascribed by the presence of acidity content represented as titratable acidity. When chia seeds are submerged in water, a mucilage layer forms within the cellular composition of the initial three layers of the chia seed coat. Once the chia seeds are fully hydrated, mucilage fibers or filaments become apparent, creating a translucent "capsule" affixed to the seed. Hydration of mucilage was significantly increased pH values by reducing the acidity (Munoz *et al.*, 2013).

Fig. 2 and Fig. 3 demonstrates how chia seeds affect titratable acidity of syrup and RTS. Titratable Acidity (TA) is an approximate of the total acidity of a solution. It can be communicated in different terms of acids. In case of syrup (Fig. 3) the lowest value (3.24%) for titratable acidity was noticed in recipe $T_8$ (80 % PJ + Fructose 45 ºBrix + 20 % CS). Whereas the highest value of titratable acidity was recorded in recipe control II $T_5$ (100 % PJ + Fructose 45 ºBrix : 3.43 %). In RTS significantly, minimum value (1.32%) for titratable acidity was noticed in recipe $T_4$ (80 % PJ + Sugar 65 ºBrix + 20 % CS ) and the highest value was recorded in recipe control II $T_5$ (100 % PJ + Fructose 45 ºBrix : 1.53%) for titratable acidity. The lowest titratable acidity is because of presence of chia seeds which are having alkaline pH increases the pH in turn reduces the titratable acidity both in RTS and syrup. The result of the present study has been widely studied in (Abdullah *et al.*, 2017; Capitani *et al.*, 2015; Balaswamy *et al.*, 2011 and Gajanana, 2002) and this might also be due to hydrolysis of polysaccharides. This effect was also observed in beverage prepared from pomegranate (Sowjanya, 2007) and in kinnow juice (Bharadwaj and Mukherjee, 2012).

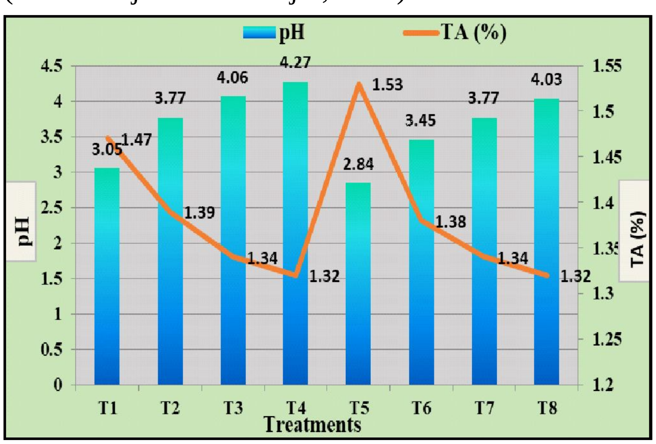


**Fig. 3:** pH and titratable acidity of chia based pomegranate RTS.

## Storage studies

Out of eight treatments, best five treatments (including controls) from organoleptic evaluation were taken for storage studies for a period of 90 days at 4ºC and were analysed for biochemical and sensorial parameters. During storage, significant difference was noticed among the treatments with respect to biochemical and sensorial parameters.

Organoleptic evaluation reveals that there was decreased trend with the progress of storage period. In Fig. 4, among the different treatments, significantly

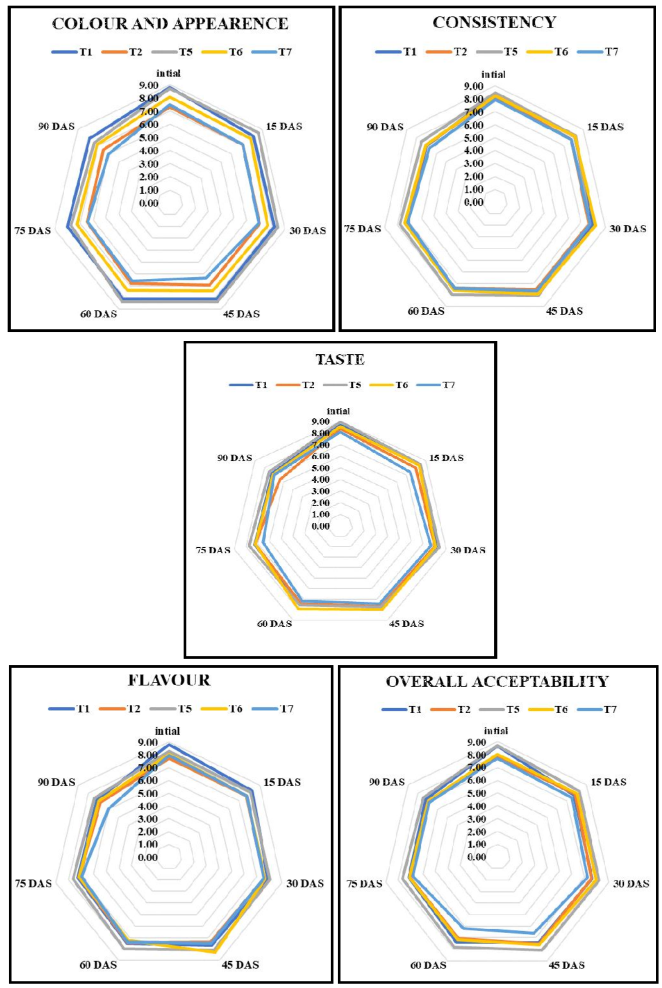


**Fig. 4:** Sensory assessment ready-to-serve (RTS) beverages prepared from pomegranate syrup with the addition of chia seeds during storage. **[$T_1$:** 100 % Pomegranate juice + Sugar (65 º Brix Control I); **$T_2$:** 90 % Pomegranate juice + Sugar (65 º Brix) + 10 % chia seeds; **$T_3$:** 85% Pomegranate juice + Sugar (65 º Brix) + 15 % chia seeds; **$T_4$:** 80% Pomegranate juice + Sugar (65 º Brix) + 20 % chia seeds; **$T_5$:** 100 % Pomegranate juice + Fructose (45 º Brix Control II); **$T_6$:** 90 % Pomegranate juice + Fructose (45 º Brix) + 10 % chia seeds; **$T_7$:** 85% Pomegranate juice + Fructose (45 º Brix) + 15 % chia seeds; **$T_8$:** 80% Pomegranate juice + Fructose (45 º Brix) + 20 % chia seeds**]**.

**Table 2:** Effect of incorporation of chia seeds on antioxidant values of the pomegranate syrup during storage (4°C).

| Treatments | Antioxidant (%) | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | Initial | 15 DAS | 30 DAS | 45 DAS | 60 DAS | 75 DAS | 90 DAS | |
| **$T_1$** | 100.31 | 98.10 | 96.02 | 92.82 | 90.25 | 87.41 | 84.26 | **92.74** |
| **$T_2$** | 112.54 | 107.58 | 104.81 | 102.10 | 98.61 | 96.88 | 93.21 | **102.24** |
| **$T_5$** | 100.90 | 97.36 | 94.45 | 92.09 | 89.46 | 86.60 | 84.27 | **92.13** |
| **$T_6$** | 108.88 | 105.86 | 103.10 | 100.90 | 98.10 | 95.79 | 93.89 | **100.93** |
| **$T_7$** | 120.57 | 115.94 | 113.39 | 109.89 | 106.93 | 105.18 | 100.42 | **110.33** |
| **Mean** | **108.64** | **104.97** | **102.35** | **99.56** | **96.67** | **94.37** | **91.17** | |
| **S.Em±** | **0.41** | **0.22** | **0.24** | **0.27** | **0.26** | **0.29** | **0.32** | |
| **CD @ 1%** | **1.64** | **0.86** | **0.96** | **1.09** | **1.03** | **1.16** | **1.31** | |

**$T_1$:** 100 % Pomegranate juice + Sugar (65 ° Brix Control I); **$T_6$:** 90 % Pomegranate juice + Fructose (45 ° Brix) + 10 % chia seeds; **$T_2$:** 90 % Pomegranate juice + Sugar (65 ° Brix) + 10 % chia seeds; **$T_7$:** 85% Pomegranate juice + Fructose (45 ° Brix) + 15 % chia seeds; **$T_5$:** 100 % Pomegranate juice + Fructose (45 ° Brix Control II)

topmost sensory score for colour and appearance was recorded in recipe $T_1$ (100 % PJ + Sugar 65 °Brix : 7.88) minimum sensory score (6.00) was recorded in recipe $T_7$ (85 % PJ + Fructose 45 °Brix + 15 % CS).

Fig. 4, indicate that during storage, sensory score for consistency was decreased, minimum score was recorded in recipe $T_7$ (85 % PJ + Fructose 45 °Brix + 15 % CS : 6.70) and highest score (7.48) for consistency was recorded in recipe $T_6$ (90 % PJ + Fructose 45 °Brix + 10 % CS). The sensory value for taste was found to be varied during the storage period. Recipe $T_5$ (100 % PJ + Fructose 45° Brix) possess highest sensory score (7.48) and the lowest (6.39) was recorded in recipe $T_2$ (90 % PJ + Sugar 65 °Brix + 10 % CS). Recipe $T_6$ (90 % PJ + Fructose 45 °Brix + 10 % CS : 7.39) found to be best for flavour and the lowest sensory score (6.00) was found in recipe $T_7$ (85 % PJ + Fructose 45 °Brix + 15 % CS). The topmost sensory score for overall-acceptability was noticed in recipe $T_6$ (90 % PJ + Fructose 45 °Brix + 10 % CS : 7.01) lowest score (6.86) was recorded in recipe $T_7$ (85 % PJ + Fructose 45 °Brix + 15 % CS).

pH of pomegranate blended syrup incorporated by chia seeds and RTS varied remarkably among the treatments. The pH value ranged from 2.71 to 4.14. As the storage period proceeds there was rise in the pH content of the pomegranate syrup based RTS incorporated with chia seeds.

Fig. 5 shows the antioxidant activity of the recipes. The antioxidant activity of syrup decreases as the storage time progress and ranged between 84.26 and 120.57 per cent (Fig. 5, Table 2). During initial days of storage (DAS), the highest antioxidant activity (120.57 %) was noticed in recipe $T_7$ (85 % PJ + Fructose 45 °Brix + 15 % CS) and significantly the lowest (100.31 %) antioxidant activity was to be found in recipe control I $T_1$ (100 % PJ + Sugar 65 °Brix). At 90 days after storage (DAS), significantly the highest antioxidant activity (100.42 %) was recorded in recipe $T_7$ (85 % PJ + Fructose 45 °Brix + 15 % CS) and the least antioxidant activity (84.26 %) recorded in recipe control I $T_1$ (100 % PJ + Sugar 65 °Brix). The antioxidant capacity of fruits and vegetables is predominantly influenced by the presence of various individual antioxidants, such as phenolic compounds, ascorbic acid, and anthocyanins, either acting independently or synergistically (Rai *et al.*, 2011).

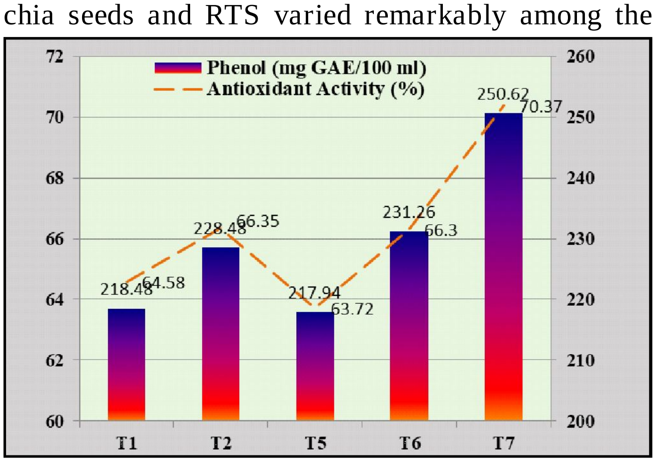


**Fig. 5:** Mean antioxidant and phenol content of chia based pomegranate RTS.

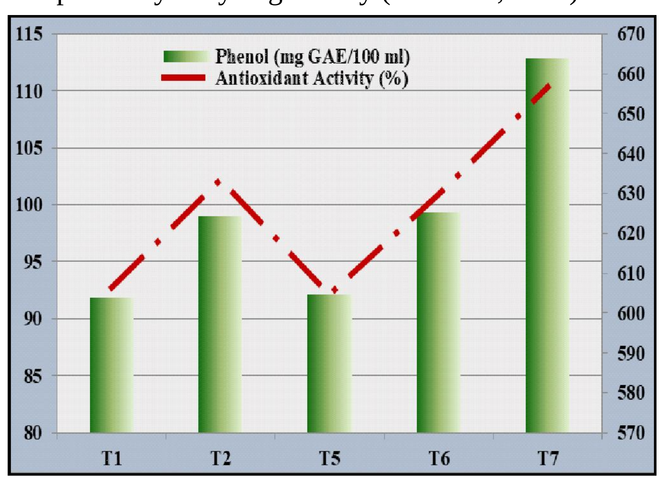


**Fig. 6:** Mean antioxidant and phenol content of chia based pomegranate syrup.

**Table 3:** Effect of incorporation of chia seeds on total phenolic values of the pomegranate syrup during storage (4°C).

| Treatments | Phenols (mg GAE/100ml) | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | Initial | 15 DAS | 30 DAS | 45 DAS | 60 DAS | 75 DAS | 90 DAS | |
| $T_1$ | 621.13 | 617.95 | 611.05 | 604.01 | 596.22 | 590.62 | 585.74 | **603.82** |
| $T_2$ | 650.75 | 643.07 | 631.50 | 623.70 | 616.54 | 606.01 | 598.27 | **624.26** |
| $T_5$ | 620.84 | 616.50 | 611.27 | 606.48 | 598.25 | 593.03 | 585.48 | **604.55** |
| $T_6$ | 653.93 | 644.71 | 632.93 | 624.74 | 614.93 | 608.03 | 596.86 | **625.16** |
| $T_7$ | 686.00 | 678.64 | 671.40 | 665.52 | 660.93 | 654.87 | 629.55 | **663.84** |
| **Mean** | **646.53** | **640.17** | **631.63** | **624.89** | **617.37** | **610.51** | **599.18** | |
| **S.Em±** | **0.22** | **0.16** | **0.37** | **0.30** | **0.24** | **0.33** | **0.45** | |
| **CD @ 1%** | **0.87** | **0.65** | **1.49** | **1.39** | **0.98** | **1.34** | **1.80** | |

**$T_1$:** 100 % Pomegranate juice + Sugar (65 ° Brix Control I); **$T_6$:** 90 % Pomegranate juice + Fructose (45 ° Brix) + 10 % chia seeds; **$T_2$:** 90 % Pomegranate juice + Sugar (65 ° Brix) + 10 % chia seeds; **$T_7$:** 85% Pomegranate juice + Fructose (45 ° Brix) + 15 % chia seeds; **$T_5$:** 100 % Pomegranate juice + Fructose (45 ° Brix Control II)

**Table 4:** Effect of incorporation of chia seeds on antioxidant values of the pomegranate syrup based RTS during storage (4°C).

| Treatments | Antioxidant (%) | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | Initial | 15 DAS | 30 DAS | 45 DAS | 60 DAS | 75 DAS | 90 DAS | |
| $T_1$ | 74.88 | 72.97 | 68.95 | 64.58 | 61.00 | 56.81 | 52.86 | **64.58** |
| $T_2$ | 77.33 | 74.08 | 70.79 | 66.57 | 63.19 | 57.90 | 54.63 | **66.35** |
| $T_5$ | 73.12 | 70.79 | 68.09 | 65.16 | 60.11 | 55.60 | 53.20 | **63.72** |
| $T_6$ | 75.82 | 73.70 | 69.38 | 67.39 | 64.02 | 58.77 | 54.99 | **66.30** |
| $T_7$ | 81.87 | 77.13 | 74.88 | 72.06 | 68.05 | 61.31 | 57.28 | **70.37** |
| **Mean** | **76.60** | **73.73** | **70.42** | **67.15** | **63.27** | **58.08** | **54.59** | |
| **S.Em±** | **0.27** | **0.20** | **0.26** | **0.20** | **0.19** | **0.17** | **0.17** | |
| **CD @ 1%** | **1.09** | **0.80** | **1.06** | **0.81** | **0.77** | **0.69** | **0.68** | |

**$T_1$:** 100 % Pomegranate juice + Sugar (65 ° Brix Control I); **$T_6$:** 90 % Pomegranate juice + Fructose (45 ° Brix) + 10 % chia seeds; **$T_2$:** 90 % Pomegranate juice + Sugar (65 ° Brix) + 10 % chia seeds; **$T_7$:** 85% Pomegranate juice + Fructose (45 ° Brix) + 15 % chia seeds; **$T_5$:** 100 % Pomegranate juice + Fructose (45 ° Brix Control II)

**Table 5:** Effect of incorporation of chia seeds on total phenolics of the pomegranate syrup based RTS during storage (4°C).

| Treatments | Phenols (mg GAE/100ml) | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | Initial | 15 DAS | 30 DAS | 45 DAS | 60 DAS | 75 DAS | 90 DAS | |
| $T_1$ | 233.04 | 228.79 | 226.41 | 218.96 | 214.13 | 208.06 | 199.98 | **218.48** |
| $T_2$ | 245.40 | 237.35 | 235.30 | 228.59 | 221.50 | 219.04 | 212.18 | **228.48** |
| $T_5$ | 235.03 | 229.03 | 220.94 | 218.28 | 212.51 | 207.93 | 201.85 | **217.94** |
| $T_6$ | 248.06 | 240.97 | 236.62 | 231.12 | 227.43 | 220.46 | 214.14 | **231.26** |
| $T_7$ | 268.49 | 260.76 | 257.02 | 250.46 | 247.09 | 240.79 | 229.73 | **250.62** |
| **Mean** | **246.00** | **239.38** | **235.26** | **229.48** | **224.53** | **219.26** | **211.58** | |
| **S.Em±** | **0.30** | **0.26** | **0.36** | **0.35** | **0.41** | **0.36** | **0.56** | |
| **CD @ 1%** | **1.22** | **1.06** | **1.46** | **1.40** | **1.65** | **1.45** | **1.96** | |

**$T_1$:** 100 % Pomegranate juice + Sugar (65 ° Brix Control I); **$T_6$:** 90 % Pomegranate juice + Fructose (45 ° Brix) + 10 % chia seeds; **$T_2$:** 90 % Pomegranate juice + Sugar (65 ° Brix) + 10 % chia seeds; **$T_7$:** 85% Pomegranate juice + Fructose (45 ° Brix) + 15 % chia seeds; **$T_5$:** 100 % Pomegranate juice + Fructose (45 ° Brix Control II)

The content of antioxidant activity of pomegranate syrup based RTS was decreased as the storage period progress and it was ranged between 52.86 and 81.87 per cent (Fig. 6, Table 3). During initial days of storage, the highest antioxidant activity (81.87 %) was recorded in recipe $T_7$ (85 % PJ + Fructose 45 ºBrix + 15 % CS) and the least antioxidant activity (52.86 %) recorded in recipe control I $T_1$ (100 % PJ + Sugar 65 ºBrix). The decline in scavenging activity could be due to the degradation of polyphenols, ascorbic acid, and flavonoid compounds over the storage period (Klimczak *et al.*, 2007 and Klimczak *et al.*, 2012) in blackberry juice. The antioxidant potential unveiled a gradual dwindle over the storage duration, ranging from 54.2 in the 10 percent sample to 60.1 in the 25 percent sample of RTS (Hirdyani, 2015). The pomegranate syrup-based ready-to-serve (RTS) beverages fortified with chia seeds exhibit elevated levels of antioxidants compared to the control sample. This could be attributed to the higher content of tocopherols present in chia seeds as reported (Kulczynski *et al*., 2019).

The reduction in antioxidant activity observed during storage may be associated with a decrease in the concentration of phenolic components and ascorbic acid in the preserved juice when compared with its fresh counterpart as reported in pomegranate squash (Karpagavalli and Amutha, 2015 and Naganagoudar, 2015). Ascorbic acid, found abundantly in fruits, serves as a potent antioxidant and significantly enhance the scavenging capacity of juices (Reddy *et al*., 2018). The antioxidant activity of the pomegranate syrup enriched with chia seeds shows higher compared to that control after three months of preservation. The similar reports were noted in cookies developed by chia seeds and quinoa seeds (Goyat *et al*., 2018) and chia fortified jamun fruit bar (Vinayaka, 2019) and in chia seed substitute bread contained approximately 6 times more total phenolic compounds 10 times higher antioxidant activity (Romankiewicz *et al*., 2017). Colour fruits and vegetables are obviously possess phenols. The total phenol content of RTS ranged between 199.98 and 268.49 mg GAE/100 ml.

Pomegranate syrup with the incorporation of chia seeds has shown significant decrease trend among the different treatments throughout three months of storage at 4 °C under refrigerated conditions (Fig. 5, Table 4). The total phenol content of syrup ranged between 585.74 and 686.00 mg GAE/100 ml. Initially, the statistic related to total phenol content was found maximum in recipe $T_7$ (85 % PJ + Fructose 45 ºBrix + 15 % CS : 686.00 mg GAE/100 ml) and after 90 days after storage, significantly, least total phenol content was recorded in recipe control II $T_5$ (100 % PJ + Fructose 45 º Brix: 585.48 mg GAE/100 ml).

Fig. 6 interprets the data collected regarding the total phenol content of ready-to-serve (RTS) beverages made from pomegranate syrup and infused with chia seeds demonstrate a gradual decrease in phenol levels over time during the storage period (Table 5). Initially, the data related to total phenol content was found maximum in recipe $T_7$ (85 % PJ + Fructose 45 ºBrix + 15 % CS : 268.49 mg GAE/100 ml) and notably minimum total phenol content was noted in recipe control I $T_1$ (100 % PJ + Sugar 65 ºBrix : 199.98 mg GAE/100 ml) after 3 months of preservation. The progressive decline in total phenols observed during the storage period may be attributed to their condensation process, resulting in the development of brown pigments, as reported in jamun squash (Kannan and Thirumanan, 2002). Also, the same reports were observed in the total phenol content of blended squash prepared from guava-jamun (Sharma *et al*., 2012). The chia seed incorporated syrup showed high phenol content compared to control samples even after 90 days after storage. This is because chia seeds are abundant in a variety of phytocompounds known for their significant biological activity. These include polyphenols such as gallic, caffeic, chlorogenic, cinnamic, and ferulic acids, as well as quercetin, kaempferol, epicatechin, rutin, apigenin, and p-coumaric acid (Oliveira-Alves *et al*., 2017 and Rahman *et al*., 2017). Similar results were reported in chia fortified jamun fruit bar (Vinayaka, 2019) and cookies developed by chia seeds and quinoa seeds (Goyat *et al*., 2012).

The minimize in the total phenol content during period of storage time was apparently as a result of polyphenol oxidase enzymatic oxidation of polyphenolic content as reported in (Altunkaya and Gokmen, 2008). The decrease in total phenol content during period of storage might be as a result of formation of precipitates with organic constituents and oxidation of phenols. Similar outcomes were reported in (Akhila, 2014 and Akhila, 2018) in jamun products. The chia seed incorporated pomegranate syrup shows high phenol content compared to control samples after 90 days after storage. Similar results were reported in chia fortified jamun fruit bar (Vinayaka, 2019) and cookies developed by chia seeds and quinoa seeds (Goyat *et al*., 2012).

## Conclusion

Pomegranate syrup containing 15 percent chia seeds exhibited significantly higher levels of antioxidants and phenolic content compared to the control sample

During storage investigations, biochemical parameters gradually declined with the progression of the storage period. However, recipe containing chia seeds maintained favourable biochemical parameters and sensory qualities for up to 90 days.

## Author Contribution

Gouthami Y- Investigation, data collection, conceived and designed the analysis, writing – original manuscript; Dr. Bhuvaneshwari G -Supervision, conceptualization, formal analysis, methodology, editing and finalization; Dr. S. L. Jagadeesh- Resources visualization, conceptualization, supervision and finalization; Dr. Chandrashekhar V. M - Methodology, resources; Dr. Anand Nanjappanavar Laborotary analysis; Dr. Vasant M. Ganige- Supervision, methodology analysis; Dr. D. L. Rudresh -Editing and writing.

## Delcarations

**Conflict of Interest:** The authors declare no conflict of interest.

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