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Article

Assessing the Protein-Ligand Interaction and Thermally Induced Quality Changes in Tomato-Based Pineapple Beverage

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Abstract: The intake of tomato in its natural form is comparatively restricted due to its limited shelf-life. Thereby, we investigated the willingness of consumers and optimized the proportions of beverages on the basis of the overall liking of the sensory panel. Further, molecular docking was also performed to evaluate the protein-ligand interactions of vitamin C, lycopene, and β-carotene against CR protein. These compounds showed great interactions with the protein targets leading to the enhancement of antioxidant activity. The most acceptable combination (S4 = 50:50 tomato and pineapple juices) was subjected to thermal processing at 70, 80, and 90 °C, respectively. Biochemical parameters such as acidity, vitamin C, non-enzymatic browning, antioxidant capacity, and total phenolics were found to be optimum in the beverage samples treated at 80 °C. It was revealed that the microbial shelf-life of beverages enhanced with an increase in processing temperatures. The untreated beverage samples could only retain a shelf-life of 4 days, however, samples treated at 80 °C for 60 s were rendered fit for 40 ± 2 days. Therefore, with the help of molecular docking, this manuscript assessed the protein-ligand interaction with the thermally induced quality changes in tomato-based beverages.

Keywords: tomato; beverage; thermal processing; refrigeration storage; shelf-life; molecular docking

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1. Introduction

Tomatoes are a rich source of nutrients providing 40% of the daily Value (DV) of vitamin C, 15% DV of vitamin A, 8% of potassium, and 7% of RDA of Iron for women and 10% RDA for men, Kumar, et al. [1]. The fruit can meet 40% of adults' body needs and two-thirds of children's daily requirements by providing 60 mg of ascorbic acid [2,3]. The phytochemical compounds in tomatoes not only promote good health but also prevent chronic degenerative disorders. Its regular consumption has also shown an inverse relationship with incidences of cancer, cardiovascular diseases, aging, and other health problems [4]. Interestingly, routine cooking of tomatoes has not been found to affect the bioavailability of these phytoconstituents present in them [5]. However, tomato fruit possesses a characteristic intense sweet-sour taste, owing to the presence of components such as reducing sugars, free acids, volatile substances, and interplay between these compounds. Apart from this, the presence of potassium and phosphate also indirectly affects the taste of the fruit [6]. Therefore, citrus fruit juices (e.g., lime) are used to mask the intense taste of tomato fruit and render the combination palatable [7,8].

Furthermore, to understand the bioavailability of the nutrients of such functional beverages by exploring the different structural conformations of compounds in the protein-ligand complex, docking studies play a prominent role. In addition, in order to explore the protein-ligand interactions for the major chemical constituents of the newly reported beverages against C-reactive protein (CRP) and human serum albumin (HSA), molecular docking studies are employed [9]. Molecular docking can aid in the identification of the amino acid residues present near the ligand at the binding site of the protein. It would also help in determining the best-fit position, which is the conformation of the ligand within the active site of the target protein. This would lead to the prediction of how a particular nutrient of the beverage or food item binds to the proteins of our body and blood and affect the functioning of that protein. Protein targets mainly CRP, whereas HSA is utilized to investigate the antioxidative benefits of various chemical compounds. CRP is generally considered the biomarker for cardiovascular disease and responsible for inducing the damage caused by oxidative stress, however, HSA, a carrier protein present in blood serum, possesses antioxidant properties. HSA has the capacity to modify its conformation by undergoing structural changes which leads to its various binding properties and redox state. Recent studies on bioactive compounds such as polyphenols, flavonoids, and phenolic acids present in beverages and various food products suggested that interactions of such compounds with CRP and HSA efficiently alter the antioxidant properties [9–11].

Commercially, the storage stability of such functional beverages is usually maintained by thermal processing at a temperature range between 50 and 150 °C or above. It is implicated that high-temperature processing is preferred for shelf-stable beverages, as higher temperature, when exposed for a smaller duration of time, kills the harmful organisms of the beverage while not causing many changes to the physicochemical properties. Most acidic juices require pasteurization, i.e., the use of temperature near 100 °C to destroy spoilage-causing organisms, while for fresh juices, a potentially effective process involves clean heating for up to 10 min at 80 °C [12,13]. Therefore, keeping these factors in mind and with the intent to utilize the maximum benefits of tomato juice, along with providing palatability, this investigation was undertaken to develop a tomato juice-based functional beverage in combination with pineapple juice with process standardization and quality evaluation. Here, tomato and pineapple juice were used to formulate a palatable shelf-stable beverage using thermal processing. First, the binding interactions were investigated for the bioactive compounds present in the newly developed tomato-based pineapple beverage with C-reactive protein and human serum albumin protein. Thereafter, the effect of thermal processing (70, 80, and 90 °C for 10 min) was studied on the quality and shelf-life of the tomato-based pineapple beverage. To the best of the author's knowledge, this is the first study to report the nutrient bioavailability using a protein-ligand interaction study of a functional beverage with a combination of tomato and pineapple juices.

2. Materials and Methods

2.1. Sample Procurement and Preparation

Fresh tomatoes were purchased from the local grain market of Patiala, Punjab, India. They were then sorted out and the damaged ones were removed. Selected tomatoes were washed, trimmed, cut, and blanched rapidly to a temperature of 70 ± 2 °C for 5 min. This was done to inactivate pectin esterase promptly and enhance juice extraction. Sliced tomatoes were then blended by using an electric blender (PHILIPS HL 1655/00) and filtered using a muslin cloth. Simultaneously, pineapple juice was freshly extracted using an electric blender (PHILIPS HL 1655/00). Both the juices (tomato and pineapple juice) were then mixed into different proportions to prepare five different samples (Table S1, Figure S1 shown in Supplementary File). The percentage of tomato juice varied between 80, 70, 60, 50, and 25%, respectively, in combination with the percentage of 20, 30, 40, 50, and 75% of pineapple juice. This was followed by the addition of 1.25% salt, 2% sugar, and 0.5% ginger juice by weight to the mix of juice (100 mL). This addition of natural additives

not only incremented the concentration of bioactive compounds in the beverage, but also rendered antimicrobial action. The mixture was then thoroughly mixed and subjected to pasteurization at 80 °C for 10 min. The beverage was allowed to cool at 20 °C in a water bath and then transferred to pre-sterilized glass bottles with a holding capacity of 30 mL. Finally, the bottles were sealed and kept at a refrigeration temperature of 4 ± 2 °C.

2.2. Sensory Evaluation

To compose a sensory panel, 45 regular consumers of fruit juices with a willingness to try a new composition were selected from an age group of 20 to 45 years. The demography of consumers and their degree of fondness and frequency of consumption of fruit juices (as 1- "Quite frequently", 2- "Once a week", 3- "Once a month", and 4- "Rarely") was enquired through a questionnaire. The selected panelists (with '1′ and '2′ frequency levels) were explained the nature of the experiment and were asked to carry out the sensory evaluation in an isolated environment at room temperature. The consumers were instructed to neutralize and clean their palate using warm filtered water before and in between each sample tasting.

2.3. Overall Liking and Food Action Rating Scale

A method similar to the one described by Ribeiro, et al. [14] was followed to assess the overall liking. The tomato-based pineapple beverage was prepared on the same day and assessed within 24 h of preparation. Different samples of beverage were coded with three-digit random numbers and were presented in a balanced monadic sequential order to avoid any no carry-over effects. Overall liking of the beverage was evaluated based on color, aroma, taste, mouthfeel, aftertaste, and overall acceptability on a 9-point hedonic scale (1 represented "dislike extremely"; 5 being "indifferent"; and 9 represented "like extremely"), and the overall liking was taken as a mean of respective values. Further, a Food Action Rating Scale—FACT (willingness to drink the beverage if it was available on the market) was also evaluated using a 5-point scale (1 represented "Would certainly not drink"; followed by 3 being "Unsure"; and 5 represented "Would certainly drink").

2.4. Thermal Processing of Beverage

Based on the scores assigned by the panelists, the beverage sample with the maximum overall acceptability score was selected and subjected to thermal treatment. The beverage samples were heated at three different temperatures of 70, 80, and 90 °C for 15 min in a thermostatic water bath, followed by their immediate cooling in an ice water bath to 20 ± 2 °C. These treated and untreated samples were then stored in pre-sterilized glass bottles at refrigeration temperatures of 4 ± 2 °C. According to the scores, the beverage sample S4, with a proportion of 50:50 tomato and pineapple juice (overall liking = 4.5 ± 0.4; FACT = 4.5 ± 0.3), was the most acceptable by the sensory panelists. While the lowest acceptability was found for the beverage sample S1 (overall liking = 2.5 ± 0.3; FACT = 2.0 ± 0.5), indicating that the overall acceptability of the beverage was least with the maximum proportion of tomato juice.

2.5. Quality Characterization

2.5.1. Total Soluble Solids (TSS)

The TSS of the sample was measured using a refractometer (LABART, EHR503). A drop of juice sample was placed on the refractometer prism and a cover plate was placed. Thereafter, the reading was noted.

2.5.2. Total Sugars

Total sugars were estimated using the Nelson-Somogyi method (for reducing) [15] and the Benedict method (non-reducing sugars) [16]. Both the values of total reducing and total non-reducing sugars were added to calculate total sugars.

2.5.3. Titrable Acidity

The titration method was used for the analysis of titratable acidity [17]. Afterwards, 10 mL of each beverage sample was diluted up to 250 mL with neutralized water. The solution was titrated against 0.1 N NaOH solution till the color of the juice became pale. Two drops of indicator (phenolphthalein) were added and titrated again until the pink color developed persistently.

2.5.4. Vitamin C

Ascorbic acid content was calculated using the 2,6 dichlorophenol indophenol method [17]. The beverage samples were first diluted with 3% meta-phosphoric acid in a 100 mL flask. Thereafter, 10 mL of this diluted sample was pipetted into a 100 mL conical flask and titrated against the dye solution until a light pink color appeared and persisted. The values were calculated in mg/100 g.

2.5.5. DPPH Antioxidant Capacity

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) is one of the most stable organic nitrogen radicals. Total antioxidant capacity was determined using the method described by Bansal, et al. [18]. Here, the beverage samples were taken and diluted with distilled water in the ratio of 1:100. In a stoppered tube, 0, 3 mL diluted beverage sample, followed by 2, 6 mL solvent (ethanol), and then 0, 3 mL of 1 mM DPPH solution were introduced and mixed properly. The absorbance of the blend was then recorded at 517 nm using a UV visible spectrophotometer (Hach DR 6000) against the blank. The values were calculated in mg/100 g.

2.5.6. Total Phenolics Content

Estimation of total phenolic content was done using the Folin-Ciocâlteu (FC) Assay [8]. The phenolic compounds get oxidized to phenolates by the reagent at alkaline pH in a saturated solution of sodium carbonate resulting in a blue complex. About 1.5 mL of FC reagent was added to 300 µL of the beverage sample, followed by the addition of 1.2 mL of aqueous 0.1 N Na_2CO_3. The mixture was allowed to stand in dark for 90 min and absorbance was read at 760 nm on a UV visible spectrophotometer (Hach DR 6000) against the blank. The values were calculated in mg/100 g.

2.6. Microbial Analysis

Sterile Petri dishes prepared with TPC agar were inoculated with 0.1 mL of untreated and each thermally treated tomato-based pineapple beverage sample, added with 0.9 mL of sterile saline water. After complete solidification, Petri dishes were inverted and placed in the incubator at 37 ± 2 °C for 24 h. The total number of colonies was counted at the end of the incubation period.

2.7. Non-Enzymatic Browning Index (NEBI)

The method by Aguiló-Aguayo, et al. [19] was employed to determine the NEBI of the untreated and thermally treated tomato-based pineapple beverage samples. To begin with, 10 mL of each beverage sample was mixed with 10 mL of ethyl alcohol (950 g L^{-1}) and centrifuged at 2000× *g* for 15 min at 18 °C. The supernatant was filtered using a 0.45 m Millipore filter and the absorbance was measured at 420 nm.

2.8. Shelf-Life

The beverage samples were subjected to microbiological analysis at an interval of 2 days. Untreated (control) and thermally treated samples of tomato-based pineapple beverages were filled in pre-sterilized glass bottles. Bottled juices were analyzed (over 46 days) for microbial counts, acidity, soluble solids, non-enzymatic browning, vitamin C, total phenolics content, and antioxidant capacity. As per the obtained values of microbial

load, retention of vitamin C and index of non-enzymatic browning, the beverage samples were analyzed for their parameters of shelf-life under safe consumption.

2.9. Molecular Docking

Molecular docking was carried out on compounds *viz*. vitamin C, β-carotene, and lycopene against C-reactive protein (PDBID: 1b09) and Human Serum Protein (PDBID: 1h9z) by employing the protein-ligand docking tool, namely Autodock 4.2.6. Biovin Discovery Studio was utilized for studying the various protein-ligand binding interactions.

2.10. Statistical Analysis

All experiments were carried out in triplicates and presented as mean ± standard deviation. Statistical significance of the results obtained from each set of experiments and the score of all the sensory attributes were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test using Minitab 19.0 software. Statistical significance was set at $p < 0.05$. Apart from this, Wilcoxon signed-rank test was applied to evaluate the overall liking and willingness to buy (FACT scale).

3. Results and Discussion

3.1. Sensory Evaluation of Tomato-Based Developed Pineapple Beverage

Sensory evaluation and profiling of tomato-based pineapple beverages were done to choose the best suitable proportion of tomato and pineapple juice to be added to develop the beverage. Therefore, in order to achieve unbiased results, a sensory panel was constituted based on their frequency of consuming fruit juices. Based on the pre-test questionnaires, it was revealed that out of a total of 46 selected sensory panelists, 32 consumed fruit juices "quite frequently" and the remaining 14 consumed "once a week". Therefore, following the consumption frequency, beverage samples with different proportions of tomato and pineapple juice (Table S1, Figure S1 as Supplementary) were evaluated for their overall liking and willingness to buy based on the FACT scale.

For overall liking, sensory evaluation of five beverage samples (S1, S2, S3, S5, S5) was carried out by the panelists and their scores were recorded on a 9-point hedonic scale. The mean scores for overall liking scores of all the samples have been plotted in Figure 1. Furthermore, since the tomato-based pineapple beverage is the first of its kind, it was important to evaluate its acceptance based on its willingness to buy. Figure 2 depicts the overall liking and FACT scores of the beverage samples. According to the scores, the beverage sample S4, with a proportion of 50:50 tomato and pineapple juice (overall liking = 4.5 ± 0.4; FACT = 4.5 ± 0.3), was the most acceptable by the sensory panelists. While the lowest acceptability was found for the beverage sample S1 (overall liking = 2.5 ± 0.3; FACT = 2.0 ± 0.5), indicating that the overall acceptability of the beverage was the least with the maximum proportion of tomato juice. The results were also supported by the data reported by Fernández-Ruiz, et al. [20] where it was found that consumers gave low scores to tomato juice having no salt, reduced sweet and tangy flavor, and fewer carbohydrates in juice. Therefore, in order to add the acidic and tangy flavor, the pineapple was mixed in different proportions in the present study owing to its advantage of being available throughout the year. Similarly, the acidic juice blends with a pH between 3.50–3.97 were found to be the most successful blends in terms of acceptability [21]. Concurrently, 60% of pineapple juice was found to be the best in the sensory score when blended with carrot juice [22]. Additionally, in our study, beverage samples with an increased percentage of tomato juice, in combination with lesser percentage of pineapple juice, achieved the lowest scores under sensory. Thereby, acidity has a major role in consumer acceptability for beverages.

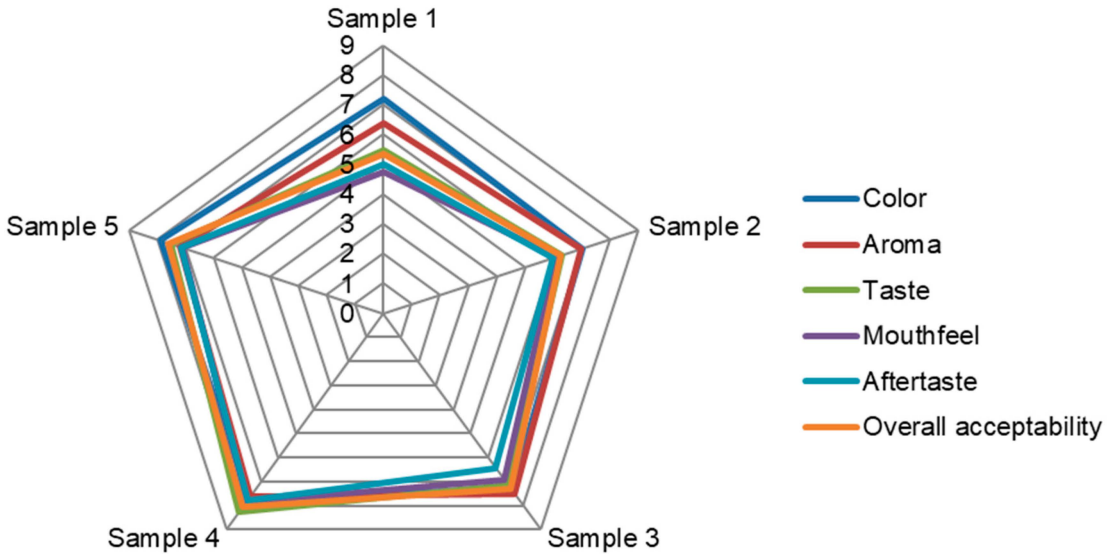


Figure 1. Overall liking scores (mean) of tomato-based pineapple beverage samples.

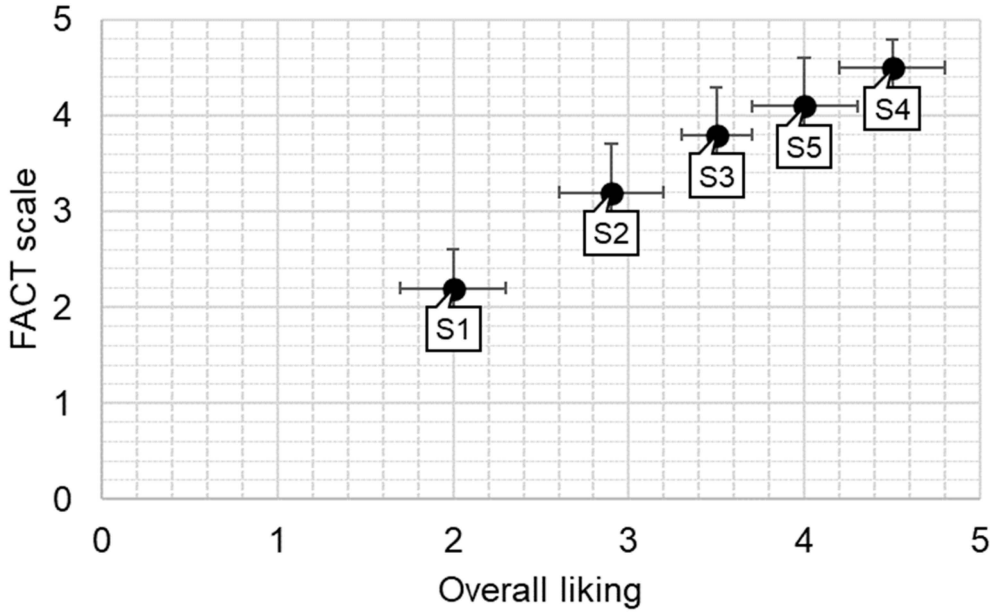


Figure 2. Evaluation of overall liking and willingness to buy (FACT scale) for tomato-based pineapple beverage samples according to Wilcoxon test applied to each variable (95% confidence). Samples S1 to S5 comprised of different concentrations of tomato juice, S1 = 80%; S2 = 70%; S3 = 60%; S4 = 50%; S5 = 25%.

3.2. Protein-Ligand Interactions through Molecular Docking

A molecular docking technique was employed to predict the best binding mode of a ligand that is a major chemical constituent of the beverages against biological targets, mainly protein. Molecular docking studies were performed for vitamin C, β-carotene, and lycopene against the C-reactive protein (CRP) (PDB ID: 1b09) and Human Serum Albumin (HSA) (PDB ID: 1h9z). Different protein-ligand poses and conformations were investigated and various interaction parameters obtained were studied. These parameters mainly included binding energy, ligand efficiency, inhibition constant, the sum of energies of Vander Waal's interactions, H-bonds, desolvation energy, and total internal energy. The stability of the protein-ligand complex is primarily indicated by the binding energy. It signifies the amount of energy released when a compound associates with the target protein. The total internal energy indicates the sum of all the energy changes that are included in

the scoring function of ligand binding at the active sites of a target protein. A high negative value for the binding and total internal energy indicates a more stable protein-ligand complex [9]. Here, the molecular docking studies assisted in obtaining the microscopic information regarding the binding interactions of drug moiety with the target protein. It is expected that the more the drug moiety interacts with the target protein, the greater the chances of deforming the structure of the protein and consecutively increasing or decreasing the efficiency of protein activity. In general, significant interactions of biologically active compounds like antioxidants with CRP decreases its activity and causes the reduction in oxidative stress, whereas with HSA efficient binding of antioxidant increases its antioxidant activity by modifying its binding mode for other drugs [23].

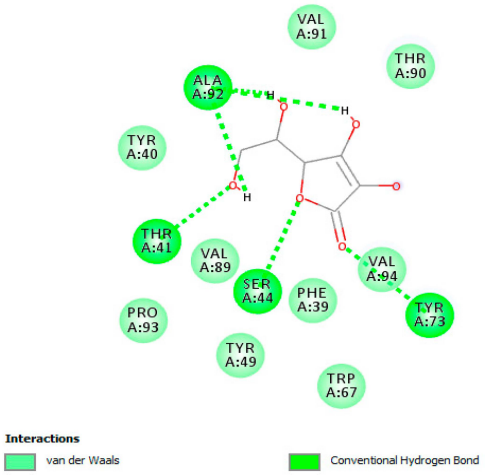
Vitamin C, β-carotene, and lycopene were docked with proteins CRP and HSA, and were found to perfectly fit in the catalytic core of the protein leading to the structural modification in it. This indicates that these compounds are interacting efficiently with the targeted proteins. Table 1 lists the various important parameters obtained from the molecular docking studies. The binding energy value for vitamin C, β-carotene, and lycopene docked with CRP was observed to be −5.66 kcal/mol, −9.16 kcal/ mol, and −2.6 kcal/mol, respectively. While the binding energy values with HSA were −5.82 kcal/mol, −2.95 kcal/mol, and −7.98 kcal/mol, respectively. These values for binding energy represent that the capacity of the interaction of these compounds is quite comparable to each other. Out of the two proteins, HSA had the maximum negative value for binding energy with vitamin C as well as lycopene, thereby suggesting greater structural modification in the protein. However, in the case of CRP, it had the most negative value for binding energy with β-carotene. Figure 3 depicts the interacting amino acids and the various bonds formed while the interaction of vitamin C, β-carotene, and lycopene with CRP and HAS, respectively. Vitamin C showed significant H-bond and vander Waal's interactions with the CRP and HSA. Whereas β-carotene and lycopene showed noteworthy vander Waal's, alkyl, and pi-alkyl interactions with both target proteins.

Table 1. Docking parameters of vitamin C, β-carotene, and lycopene.

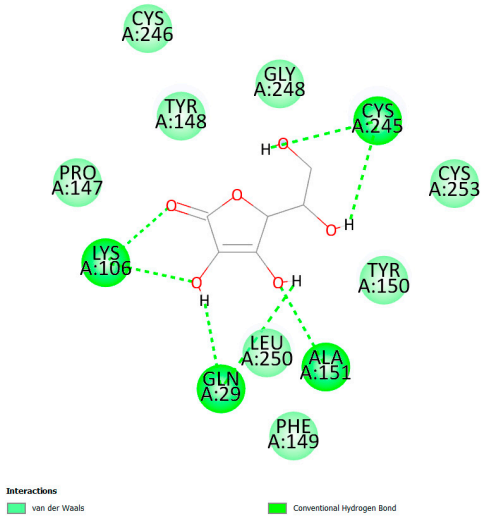
S. No.	Compound	Binding Energy (kcal/mol)	Vdw_hb_ desolv_energy	Total Internal Energy	Ligand Efficiency	H-Bonds	Vander Waals Interaction
			Vitamin C				
1	VC, 1b09	−5.66	−7.27	−3.13	−0.47	THR A:41 THR A:90 ALA A:92	PRO A:93 TYR A:40 VAL A:91 VAL A:94 VAL A:89 SER A:44 TYR A:49 PHE A:39
2	VC, 1h9z	−5.82	−7.15	−2.85	−0.49	LYS A:106 GLN A:29 ALA A:151 CYS A:245	CYS A:246 GLY A:248 CYS A:253 TYR A:150 PHE A:149 LEU A:250 PRO A:147 TYR A:148

Table 1. *Cont.*

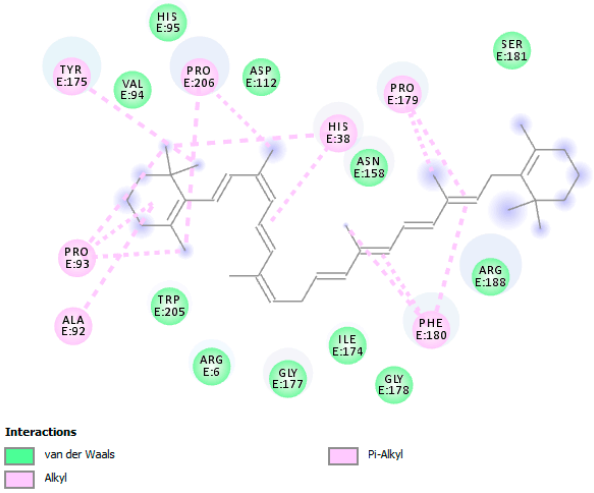
S. No.	Compound	Binding Energy (kcal/mol)	Vdw_hb_ desolv_energy	Total Internal Energy	Ligand Efficiency	H-Bonds	Vander Waals Interaction
				β-carotene			
3	BC, 1b09	−9.16	−12.15	−1.71	−0.23	HIS E:95 VAL E:94 ASP E:112 ARG E:6 GLYE:178 SER E:181 ARGE:188 GLYE:177 GLYE:177 ASNE:158 ILE E:174 TRP E:205	TYR E:175 PRO E:93 ALA E:92 HIS E:38 PRO E:206 PHE E:180 PRO E:179
4	BC, 1h9z	−2.95	−5.92	−1.66	−0.07	ASP A:108 CYS A:246 GLN A:104 GLU A:100 GLU A:97	HIS A:247 LEU A:103
				Lycopene			
5	Lyco, 1b09	−2.6	−11.25	−2.78	−0.07	LEU E:204 TRP E:205 ASP E:112 LEU E:176 ILE E:174 ASN E:158 ARG E:188 SER E:5 ALA E:92 HIS E:95 GLY E:177 ARG E:6 GLY E:178 PRO E:179	PRO E:93 PRO E:206 PRO A:115 LYS A:114 HIS E:38 TYR E:175 PHE E:180
6	Lyco, 1h9z	−7.98	−13.35	−1.74	−0.2	SER A:193 GLN A:196 GLY A:248 SER A:192 GLU A:292 GLU A:153 ARG A:160 GLU A:188	HIS A:146 TYR A:148 ARG A:197 PHE A:149 CYS A:200 CYS A:245 CYS A:253 TYR A:150 ARG A:257 CYS A:246 VAL A:241 LYS A:195 PHE A:157 HIS A:288 PHE A:156



(**a**) VC, 1b09

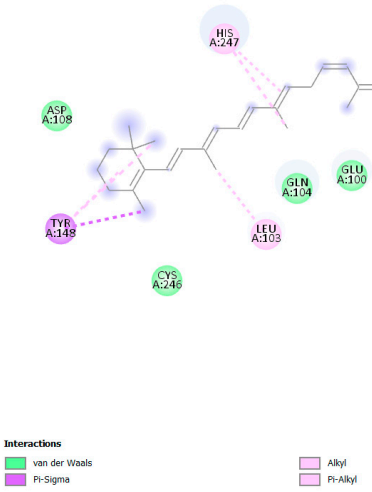


(**b**) VC, 1h9z

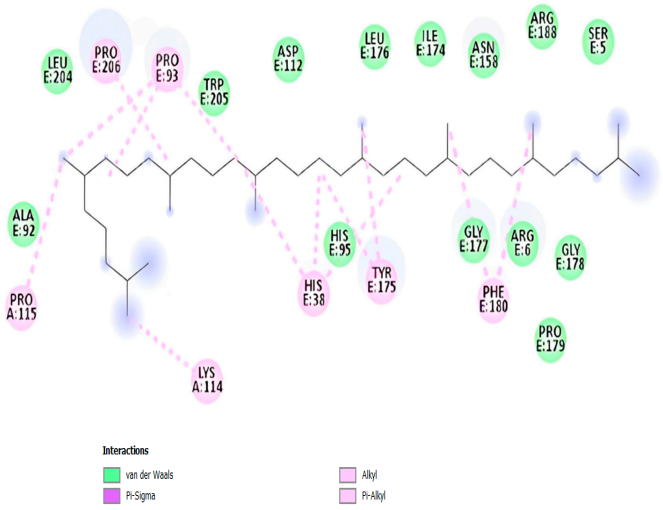


(**c**) BC, 1b09

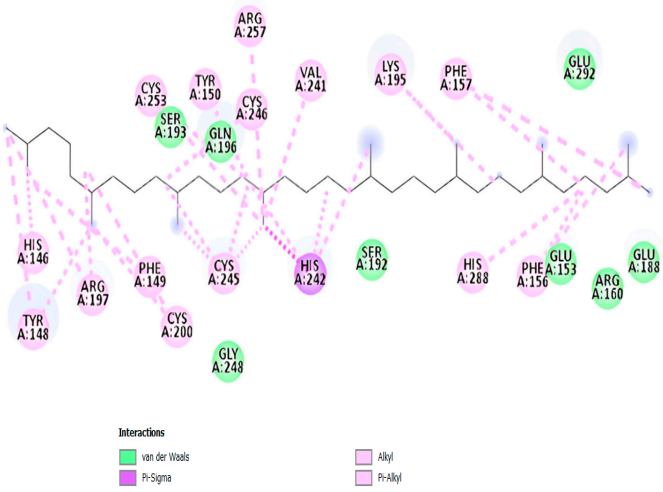
Figure 3. *Cont*.



(**d**) BC, 1h9z



(**e**) Lyco, 1b09



(**f**) Lyco, 1h9z

Figure 3. 2D Protein-ligand binding interactions map for Vitamin C (**a**,**b**), β-carotene (**c**,**d**), and lycopene (**e**,**f**) docked with proteins.

The compounds present in the beverage are well known for their therapeutic effects. High levels of CRP indicate inflammation due to many unwanted conditions. It is also an important part of innate immunity in our body as it activates macrophages and oxidative stress. It is the indicator of inflammation in our body, and therefore, the elevated levels of CRP proteins indicate the risk for cardiovascular problems and other infections from diseases such as cancer [24,25]. On the other hand, the protein HSA is synthesized in the liver and found in the blood. It is also associated with anti-inflammatory properties in the body and lower levels of HSA indicate the risk of developing cardiovascular diseases. Apart from carrying endogenous and exogenous substances, it is an important antioxidant of blood and helps in maintaining blood pH levels [9]. As evident in the present study, the bioactive compounds present in the beverages, mainly vitamin C, β-carotene, and lycopene, are significantly interacting with the amino acids of the target protein as these compounds perfectly fit at the binding site of the proteins. This suggests that the interaction of these compounds has decreased the activity of CRP and increased the antioxidant efficiency of HSA, therefore, beverages reported in this study possess therapeutic potential.

3.3. Effect of Thermal Treatment on the Quality Characteristics of Beverage

The TSS and total sugars of tomato-based pineapple beverage samples treated at different temperatures did not show any significant ($p \leq 0.05$) changes. The TSS and total sugars of beverage samples untreated and treated at 70–90 °C ranged from 14.50 to 14.80% and 12.40 to 12.48 g, respectively (Figure 4). These results were found to be similar to the inferences of the study conducted by Mehta, et al. [26] and Caminiti, et al. [27], where no significant ($p \leq 0.05$) changes were observed in TSS values of thermally processed tomato-based beverages. Ordóñez-Santos, et al. [28] stated that this non-significant ($p \leq 0.05$) change could be due to the intensity or energy applied, which resulted in no effect on the physiochemical properties of the high molecular weight structures.

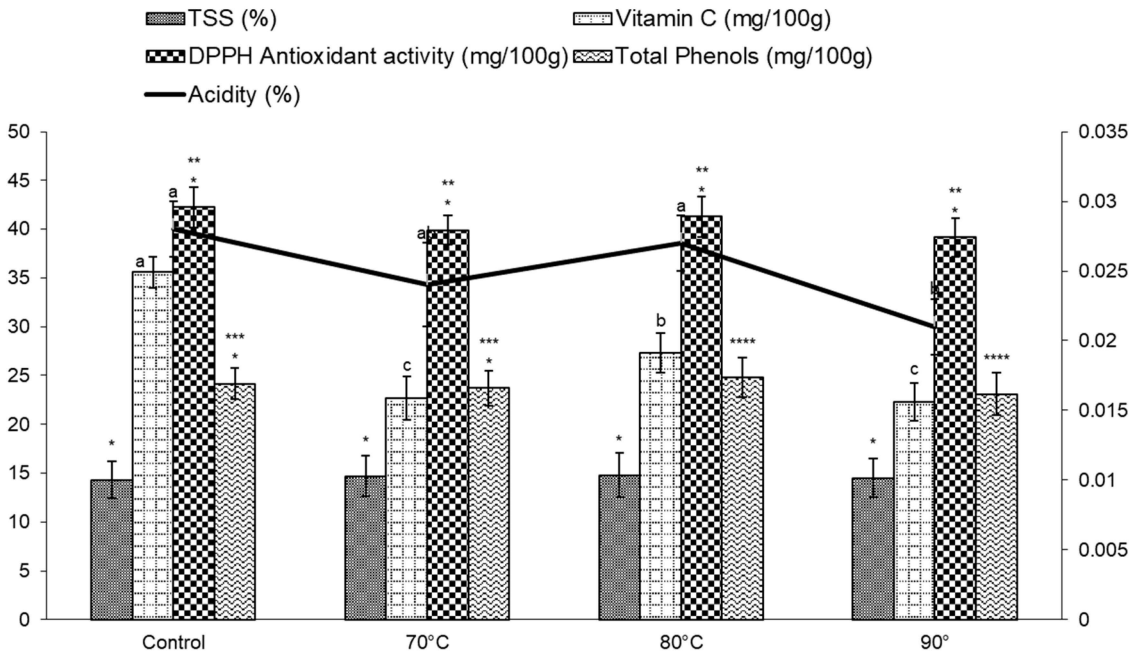


Figure 4. Vitamin C content, DPPH antioxidant activity, and total polyphenolic content of tomato-based pineapple beverage samples thermally treated at 70, 80, and 90 °C. Different number of stars and lower-case alphabets represents significant difference in the values for samples untreated and treated at different temperatures.

Similarly, the acidity of tomato-based pineapple beverages untreated and treated at different temperatures (70, 80, and 90 °C) ranged between 0.021 and 0.028%, as presented in Figure 4. Similar to TSS and total sugars, acidity did not show any significant effect

($p \leq 0.05$) of thermal treatments on the beverage samples. Astuti, et al. [29] studied the effect of ohmic heating (at 70, 80, and 90 °C) on tomato juice for 15, 30, and 45 min and obtained contrary results. It was seen that high temperature yielded a higher acidity, and increased heating duration tends to result in a lower pH of the tomato juice. According to Chia, et al. [30], total acidity is related to organic acids present in fruits and vegetables, leading to a decrease in the pH of the beverage at higher concentrations of organic acids. The heating of tomato juice has been found to increase ascorbic acid degradation and pyroglutamic acid formation, with no effect on citric acid and malic acid [31]. However, heating pineapple juice at 75, 85, and 95 °C for 15 min had a minimal effect on ascorbic acid degradation, as compared to treatment for prolonged periods [32]. Thus, in the present study, no significant difference in the acidity of the beverage might be due to the minimal degradation of ascorbic acid, owing to short heat treatment times.

Another quality indicator in terms of vitamin C, whose presence also signifies the nutritional content of the beverage, is shown in Figure 4 at different temperatures. Amongst the tomato-based pineapple beverage samples treated at 70, 80, and 90 °C, the maximum retention of vitamin C was reported in the beverage sample treated at 80 °C (27.3 ± 2.2 mg/100 g) after the control beverage sample (35.6 ± 1.6 mg/100 g) which is the maximum beyond 50% of the original sample. However, no significant ($p \leq 0.05$) difference was found in the vitamin C content of beverages treated at 70 and 90 °C (22.7 ± 1.6 and 22.3 ± 1.1 mg/100 g, respectively). In the case of orange juice, it has been reported that the application of higher temperatures (70–85 °C) leads to the breakdown of ascorbic acid [33,34]. Khare, et al. [35] reported a similar trend in sugarcane juice and stated that this reduction might be due to the oxidation of ascorbic acid into dehydrated ascorbic acid.

Furthermore, the functional quality of the beverage was assessed with the concentration of total polyphenols and antioxidant activity. Polyphenols are the secondary metabolites found in plants comprising redox properties responsible for antioxidant activity [8,18]. The total polyphenolics of tomato-based pineapple beverage samples treated at different temperatures are presented in Figure 4. Similar to the DPPH antioxidant activity values, the total phenolics were also not altered with thermal processing. Total phenolics were recorded in the range of 23.10 to 24.78 mg/100 g. The results revealed that total phenolics, as well as the antioxidant activity of the beverage samples, were retained irrespective of the thermal processing conditions. Similarly, Mehta, Sharma, Bansal, Sangwan, and Yadav [26] also stated that the total phenolic activity was retained in the thermally treated tomato-based beverage. In addition, no significant ($p \leq 0.05$) change was reported in the total phenolic content of tomato-based beverages, despite increasing the thermal processing time from 10 to 15 min [2]. This could be due to the cell wall disruption at higher temperatures, leading to higher extractability of bound phenolics and simultaneous degradation of polyphenol compounds [36].

However, thermally treated tomato-based pineapple beverage samples showed a non-significant ($p \leq 0.05$) difference in DPPH antioxidant activity. The results (Figure 5) depicted that the antioxidant values of all beverage samples, including control and those treated from70 to 90 °C, ranged between 39.15 and 42.25 mg/100 g. In a study conducted by Vervoort, et al. [37], similar results were obtained in thermally processed orange juices, i.e., no significant ($p \leq 0.05$) changes were reported in antioxidant properties. The authors suggested that the formation of compounds under the Maillard reaction might have led to the non-significant ($p \leq 0.05$) change in the antioxidant capacity.

3.4. Shelf-Life Evaluation Using Microbiological Analysis

The data with respect to NEBI, TPC, and Vitamin C, which were considered the major deciding factors to establish the shelf-life of tomato-based pineapple beverage samples untreated and treated at different temperatures, are presented in Figures 5 and 6**.**

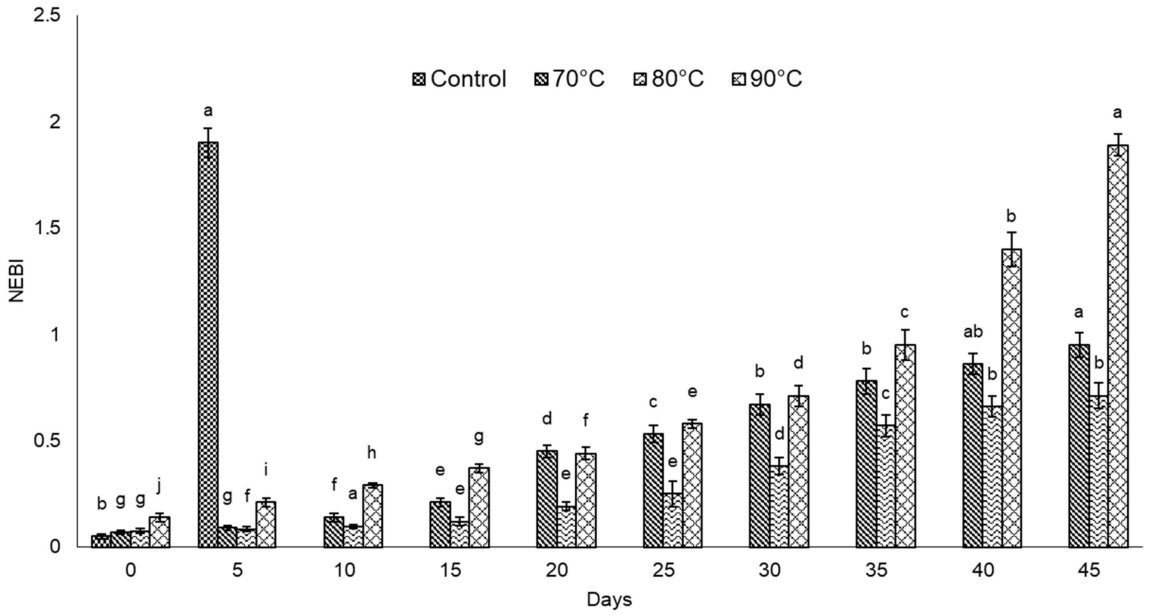


Figure 5. Non-enzymatic browning index (NEBI) of tomato-based pineapple beverage samples thermally treated at 70, 80, and 90 °C during storage. Different lower-case alphabets represents significant difference in the values at different storage period.

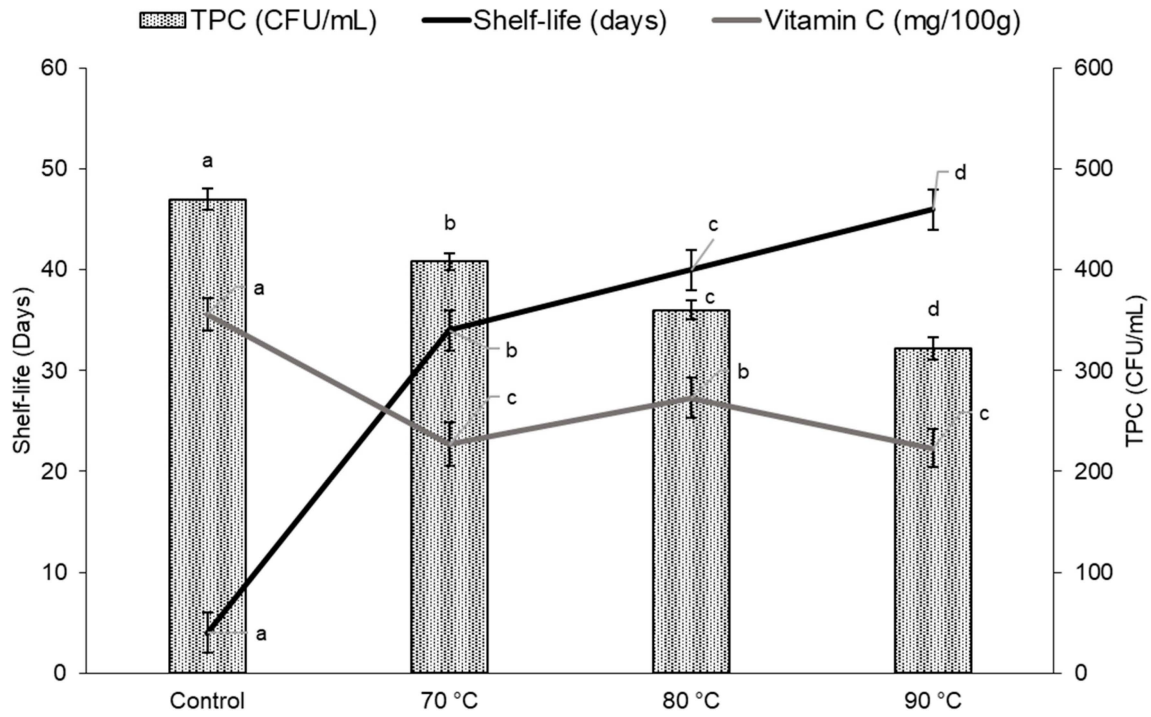


Figure 6. TPC and shelf-life of tomato-based pineapple beverage samples thermally treated at 70, 80, and 90 °C. Different lower-case alphabets represents significant difference in the values for samples untreated and treated at different temperatures.

3.4.1. Non-Enzymatic Browning Index (NEBI)

Non-enzymatic browning is a barometer of quality index that showcase the sensory characteristics and the enhanced absorbance values show the development of brown color over the period of storage. Figure 5 shows the effect of heat treatment conditions on the NEBI of beverages during storage till 45 days. The absorbance value of the untreated beverage sample was 0.12 at the 0 days and became spoiled by the end of the mere third

day with the attainment of a NEBI value of 1.9 ± 0.17. However, among the thermally treated beverage samples, the samples treated at 90 °C attained the value of 1.4 ± 0.22 at the end of the storage of 40 days in comparison to the absorbance value of 0.66 ± 0.03 of the beverage treated at 80 °C with the period of similar refrigeration storage. A persistent increase in browning was found in all the thermally treated beverage samples throughout the storage period, as on the application of heat, the phenolic compounds form complexes with proteins that have darkened the beverage. Similar results of browning were found in the thermal processing of orange-juice-milk beverage (90 °C for 15, 21 s and 98 °C for 15, 21 s) [38] and whey-based lime beverage (90 °C for 60 s) [8]. Similarly, the conditions of pre-pasteurization (98 °C, 30 s) and pasteurization of apple juice resulted in immediate darkening that increased the turbidity to the value of 1.31 ± 0.04 and 1.87 ± 0.08 [39]. However, the beverage samples treated at lower temperatures (70 and 80 °C) relative to 90 °C achieved significantly less turbidity (0.91 ± 0.21 and 0.66 ± 0.11) as lower temperature decreases the rate of particle dissociation [40]. Therefore, the extent of the browning depends upon the conditions of the treatment employed. Additionally, the temperatures and times of treatment need to be continually monitored.

3.4.2. Total Plate Count (TPC)

A decrease in microbial load in beverage samples was observed with an increase in thermal processing temperatures (Figure 6). TPC values for beverage samples thermally treated at 70, 80, and 90 °C increased from 72 ± 8.0 CFU/mL at 0 days to 408 ± 8.8 CFU/mL at 34 ± 2 days, 360 ± 9.7 CFU/mL at 40 ± 2 days and 322 ± 11.3 at 46 ± 2 days CFU/mL, respectively. However, the control sample had the highest TPC value of 470 ± 10.9 CFU/mL (at a very short span of only 4 days). Since the TPC values were $\geq 10^3$ on the consecutive day of microbial analysis, therefore, further analysis was discontinued, and the shelf-life of the beverage samples was reported (Figure 6). Similar results were reported by Jan and Masih [22], who stated that a heat treatment of pineapple juice blend at 90 °C was more effective for inactivating the microbial flora, with a substantially extended shelf-life of 21 days. However, the enhanced shelf life of 40–45 days in the present study might be due to the addition of a natural additive of ginger that may have rendered the antimicrobial action. Similarly, Mena, et al. [41] also found that higher thermal temperatures supported the shelf life of pomegranate juices and that HTST treatment imparted a significant ($p \leq 0.05$) protective and preservative role as compared to LTST. Recently, various other studies have also reported a similar increase in the shelf-life of beverages with thermal treatment. For instance, wood apple beverages- from 8–12 h to 50 days when pasteurized at 85 °C for 10 min [42]; litchi and beetroot juice beverage- 90 days when pasteurized at 100 °C [43].

4. Conclusions

In the present study, a sensory-optimized tomato-based pineapple beverage was studied for its protein-ligand binding interactions using molecular docking studies. The study revealed that bioactive compounds present in the beverage significantly interacted with the amino acids of the target protein and hence suggested enhanced antioxidant potential. Major interactions observed between protein and antioxidant compounds were hydrogen bonding, pi-alkyl, alkyl-alkyl, and van der Waals interactions. Furthermore, the thermally processed tomato-based beverage (at 80 °C for 10 min) with the composition of 50% tomato and 50% pineapple juice was found to be the most acceptable in terms of biochemical properties and microbial shelf-life with respect to thermal treatment at 70 and 90 °C. Based on the processing temperature, beverage samples treated at 70, 80, and 90 °C achieved microbial shelf life of 35 ± 2, 40 ± 2, and 45 ± 2 days, respectively. However, the beverage treated at 80 °C was found to be the best in terms of retaining the nutritional quality and microbial shelf-life.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages9010012/s1, Figure S1: Tomato-based pineapple beverage samples; Table S1: Presentation of tomato-based pineapple beverage samples developed for the study.

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