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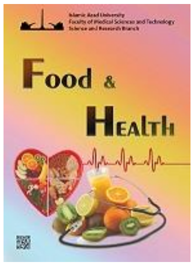

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Epsilon-Polylysine: A Substitute for Pasteurization in Fruit and Vegetable-Based Beverages and Its Effects on Physicochemical, Microbiological, and Organoleptic Properties

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ABSTRACT

This study aimed to investigate the potential of epsilon-polylysine as a substitute for pasteurization in beverages derived from a mixture of fruit and vegetable juices. Pasteurization is widely employed in the food industry as the primary method to inactivate microorganisms and enzymes in beverages. In contrast, epsilon-polylysine is known for its non-toxic, cationic properties and proven antimicrobial efficacy. To achieve this objective, different concentrations of epsilon-polylysine (0.025, 0.05, 0.075, and 0.1%) were added to the beverage formulation. Throughout a 28-day storage period at 4°C, a comprehensive evaluation was conducted on physicochemical properties (pH, titratable acidity, soluble solids, total sugar, formalin number, viscosity, vitamin C content, and color indices), microbiological parameters (total microbial count, mold, and yeast count), and sensory attributes (taste, color, aroma, and overall acceptance). Results indicate that pasteurization leads to decreased Brix value, formalin number, and vitamin C concentration compared to unpasteurized treatments ($p<0.05$). Pasteurized beverages also exhibit a darker and more reddish hue, indicating a decrease in yellow chromaticity. However, pH, acidity, total sugar, and viscosity values of the beverages remain unaffected by pasteurization. Microbiological evaluations indicate that formulations containing 0.075% and 0.1% epsilon-polylysine exhibit comparable outcomes to pasteurization, successfully inhibiting the proliferation of bacteria, molds, and yeasts during the storage period. Sensory evaluations show that incorporating varying levels of epsilon-polylysine has no negative impact on the sensory attributes of the beverages, maintaining their appeal throughout storage. In conclusion, the findings demonstrate that epsilon-polylysine, particularly at higher concentrations (0.075 and 0.1%), can effectively maintain the quality of fruit and vegetable-based beverages while showing promise as a viable substitute for the pasteurization method.

1. Introduction

Fruit and vegetable juices play a crucial role in promoting a healthy diet, with their consumption highly recommended for obtaining essential nutrients. These natural products are low in fat and rich in vitamins, minerals, and dietary fiber, making them valuable sources of nourishment. They notably contain substantial vitamin C content and exhibit significant antioxidant capacity, thus contributing to the mitigation of oxidative stress and potentially reducing risks associated with conditions such as cardiovascular diseases, hypertension, diabetes, and various cancers (1). In recent years, the popularity of fruit-based beverages has increased, attributed to their appealing flavor, superior nutritional profile, and diverse health benefits (2). Microbial spoilage of food products remains a primary concern within the food supply chain, resulting in substantial financial losses and health concerns due to the proliferation of pathogenic microorganisms or the

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production of harmful toxins. Among consumables, fruit juices provide an optimal environment for bacterial growth. Similarly, fungi find favorable conditions within fruit juice environments, emerging as prevalent agents of spoilage in such products (3, 4). Fruit juices commonly undergo a heat treatment process called pasteurization, typically performed at temperatures ranging from 88 to 94°C for durations of 15 to 45 seconds (5). The primary objective of pasteurization is to inactivate thermophilic microorganisms responsible for food spoilage and pathogens such as molds, yeasts, and vegetative bacteria (6). Beyond microbial inactivation, thermal pasteurization effectively neutralizes enzymes present in beverages, which are responsible for oxidative transformations during processing and storage, as well as modifications in the color of fruit-based drinks (7). Consequently, heat treatment of fruit juices extends their shelf life while minimizing quality degradation (8). However, applying heat treatments can lead to undesired changes in the functionality of heat-sensitive bioactive compounds and the sensory attributes of products, including color, taste, and aroma (9, 10). As a result, efforts are being directed towards replacing heat-based pasteurization methods with non-thermal processes (11). Epsilon-polylysine has gained recognition as a highly potent antimicrobial agent for food preservation (12). This compound, characterized as a homopolymer of the amino acid lysine, features a side chain length ranging between 25 and 35 units. Produced through aerobic fermentation by the bacterium *Streptomyces albulus*, this polypeptide is widely used as an effective antimicrobial agent, particularly in Japan and the United States (13). Extensive research underscores the substantial antimicrobial efficacy of polylysine against a diverse array of microorganisms, including both gram-positive and gram-negative bacteria, molds, and yeasts (14). Epsilon-polylysine's mode of action involves electrostatic binding to the cell surface, thereby impeding outer membrane formation and disrupting normal cytoplasmic distribution (15). This compound exhibits antimicrobial activity across a broad pH spectrum and remains non-toxic to humans even at elevated concentrations, exerting antimicrobial effects even at minute levels (16). Notably, in 2004, epsilon-polylysine received designation as a generally recognized as safe (GRAS) substance for food application from the United States Food and Drug Administration (17). Given the established antimicrobial properties of epsilon-polylysine demonstrated in prior research, the primary focus of the present study was to investigate the potential of substituting heat pasteurization with epsilon-polylysine as a strategy to extend the microbial shelf life of beverages obtained from a mixture of fruit and vegetable juices.

2. Materials and methods

2.1. Materials and chemicals

Epsilon-polylysine was obtained from HANDARY (Germany). Fruit concentrates and purees were sourced as follows: pear concentrate from Konfrut (Turkey), apple concentrate and apple puree from TATAO (Iran), carrot concentrate from AURELLI (Italy), and lemon concentrate from DOHLER (Turkey). Citric acid was acquired from JIANGJO (China).

2.2. Samples preparation

The base formulation of the composite beverage is outlined in Table 1. Initially, 50% of the prepared water was added to the container to create the beverage treatments. Subsequently, a mixture of apple concentrates and apple puree was combined and incorporated into the blending vessel. A series of additives including pear concentrate, carrot concentrate, ginger extract, and lemon concentrate were then successively added to the mixture. A high-velocity blending procedure was performed for 20 minutes. Following this, a solution of citric acid dissolved in water was independently introduced into the mixing tank. The control sample was generated through heat pasteurization, exposing the beverage to a temperature of 96°C for 30 seconds. Epsilon-polylysine was incorporated at four concentrations (0.025%, 0.05%, 0.075%, and 0.1%) to formulate unpasteurized treatments within the beverage mixture. Fig.1 illustrates the control beverage, subjected to pasteurization, alongside the unpasteurized beverage enriched with 0.1% epsilon-polylysine.

Table 1. Formulation of basic beverage.

Ingredients	Amounts (%)
Pear concentrate	2.7
Apple concentrate	7.8
Apple puree	12.0
Carrot concentrate	5.0
Lemon concentrate	4.5
Ginger extract	0.015
Citric acid	0.11
Water	67.875

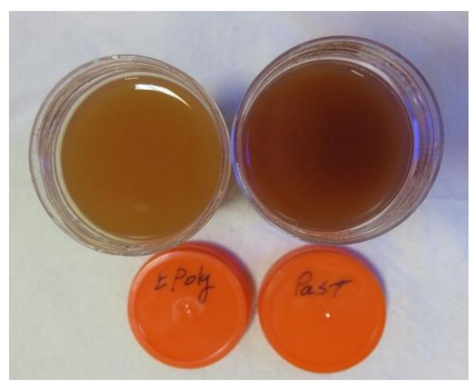

Fig. 1. Pasteurized and non-pasteurized (containing Ɛ-PL) beverages based on mixture of fruits and vegetables juices

2.3. pH Measurement

The pH levels of the beverage treatments were measured according to the Iranian National Standard No. 2685, using a pH meter (METROHM, Switzerland). The instrument's electrode was directly immersed into the sample (Iranian National Standard, 1992).

2.4. Titratable acidity

Titratable acidity of the beverages was measured in accordance with the Iranian National Standard No. 2685, with results expressed as citric acid content (Iranian National Standard, 1992). A 20-gram sample containing phthalic acid was titrated using 0.1 N sodium hydroxide solution. The following equation was used to calculate titratable acidity:

$$\text{Acidity (g/100g)} = \frac{\text{Titrant volume} \times 0.0064 \times 100}{\text{Sample weight}}$$

2.5. Soluble solid content (Brix)

Soluble solid content in beverages, measured in Brix units, was analyzed using a refractometer (ATAGO, Japan), following the guidelines stipulated by the Iranian National Standard No. 2685, at a controlled temperature of 20°C (Iranian National Standard, 1992).

2.6. Total sugar content

Total sugar content was determined as follows: In a 100 ml volumetric flask containing 25 ml of clarified solution, 6-10 ml of hydrochloric acid (3 + 1) was added. The flask was placed in a 70°C water bath for 10 minutes. After cooling, a few drops of phenolphthalein indicator were added, followed by titration with sodium hydroxide solution (40%) and 0.1 N sodium hydroxide solution until a faint pink color appeared. After confirming color stability, the solution was diluted to volume with distilled water, creating solution J. In a 250 ml Erlenmeyer flask, 5 ml each of Fehling A and B solutions were combined. Glass boiling stones, 3-4 drops of methylene blue indicator, and approximately 20 ml of distilled water were added to prevent excessive evaporation. The mixture was heated to boiling and maintained for 2 minutes. Solution J was then gradually added from a burette to the boiling Fehling solutions until the blue color disappeared, resulting in a reddish brick-red color indicative of Cu2O formation. The volume of solution consumed was recorded. Total sugar content was calculated using the following equation, where F represents the Fehling factor according to the Iranian National Standard (1992):

$$\text{Total sugar (g/100g)} = \frac{\text{F} \times 100 \times 100 \times 100}{1000 \times \text{volume} \times 25 \times 25}$$

2.7. Formalin Number Measurement

To assess the formalin number in beverages, 25 ml of the sample was placed in a burette positioned on a magnetic stirrer. 1.0 N sodium hydroxide was added to achieve a pH of 8.1. Following the addition of 10 ml neutral formaldehyde, the solution was stirred for one minute. 0.25 N sodium hydroxide was then added dropwise until pH 8.1 was reached. The volume of sodium hydroxide used was recorded. The formalin number was calculated using the following equation, as per the Iranian National Standard (1992):

$$\text{Formalin number} = \frac{\text{Titrant volume} \times 0.25 \times 10 \times 100}{\text{Sample volume}}$$

2.8. Viscosity measurement

Viscosity measurements were conducted using a Brookfield DV-II-PRO viscometer (USA). Spindle number 60 was selected based on preliminary tests. All assessments were performed at 20°C. Viscosity readings were obtained at 50 rpm, 15 seconds after spindle rotation (18).

2.9. Vitamin C measurement

To quantify vitamin C content, 5 g of sample was mixed with 20 ml of 3% metaphosphoric acid solution and left at room temperature for 20 minutes. After filtration, 5 ml of the resulting solution was titrated with 2,6-dichlorophenol indophenol solution until a pink color appeared. Vitamin C content was calculated using the following equation (19):

$$\text{Vit C} = \frac{\text{e} \times \text{d} \times \text{b}}{\text{c} \times \text{a}} \times 100$$

Where, e is the volume of 2,6-dichlorophenol indophenol solution consumed, d is the color factor (amount of color solution used for standard titration divided by 0.5), b is the volume of metaphosphoric acid, c is the volume of solution used for titration, and a is the sample weight.

2.10. Color parameters measurement

Color characteristics were assessed using a colorimeter (KONICAMINOLTA, Japan). 15 ml of beverage was placed in a 50 ml glass container, and color attributes were measured, including L* (lightness), a* (red-green intensity), and b* (yellow-blue intensity). Overall color difference (ΔE) was calculated using the following equation (20):

$$\Delta E = \sqrt{\left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2 + \left(L^* - L_0^*\right)^2}$$

2.11. Microbiological Tests

Total microbial counts were determined using the mixed culture technique as per the National Standard Organization of Iran, document number 5272-1. Plate Count Agar (PCA) medium was used, with plates incubated at 30°C for 72 hours. Colonies were then enumerated (National Standard Organization of Iran, 2014). Mold and yeast enumeration followed the National Standard Organization of Iran, document number 10899-1, using surface plating on Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar). Plates were incubated at 25°C for 5 days before examination (National Standard Organization of Iran, 2016).

2.12. Sensory evaluation

Sensory characteristics (taste, color, aroma, and overall acceptability) were evaluated using a 5-point Hedonic scale: 1: Not consumable or very bad; 2: Unacceptable or bad; 3: Acceptable or average; 4: Satisfactory or good; 5: Highly satisfactory or very good.

2.13. Data analysis

Experiments were conducted using a completely randomized design with three replications. Results were analyzed through one-way analysis of variance (One-way ANOVA) using SPSS version 22. Treatment means were compared using Duncan's multiple range test at a 95% confidence level ($p<0.05$). Graphical representations were generated using Excel software. The measure of titratable acidity is employed as an indicator reflecting the overall concentration of acidic compounds present.

3. Results and Discussion

3.1. pH values of fruit and vegetable-based beverages

Fig. 2 illustrates the pH values of fruit and vegetable-based beverages over a 28-day refrigerated storage period. Initially, pH values of different beverage treatments ranged from 3.37 to 3.39. On day 0, no statistically significant differences were observed between the pasteurized sample (control) and treatments containing various levels of epsilon-polylysine ($p>0.05$). During refrigerated storage, a gradual decrease in pH was noted, attributable to bacterial activity, sugar consumption, and organic acid formation (e.g., lactic acid). No significant differences in pH values were observed among beverage treatments on the second day of storage. However, by the final day, the control sample exhibited the lowest pH (3.28), followed by the 0.025% epsilon-polylysine treatment (pH 3.35). The 0.05% epsilon-polylysine treatment displayed the highest pH (3.34), though no statistically significant differences were observed between this treatment and those containing 0.075% and 0.1% epsilon-polylysine.

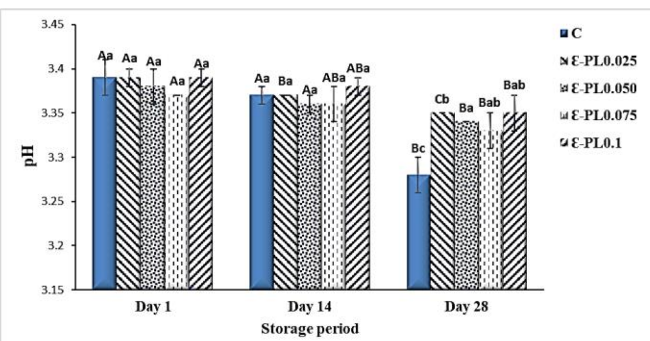

Fig.2. Changes in pH values of beverage treatments during the 28-days storage period at 4 °C. Different small and big letters represent significant difference at 5% level of probability among samples and storage period, respectively. C: control (pasteurized sample); Ɛ-PL: unpasteurized sample containing Ɛ-Polylysine.

Roufegarinejad et al. (21) reported a gradual decrease in apple juice pH over time. Nayak et al. (22) and Vollmer et al. (23) documented limited pasteurization effects on apple and pineapple juice pH. Ferreira et al. (24) noted minimal initial pasteurization effects on prickly pear fruit juice pH, with a decline observed during 40-day storage. Emelike and Obinna-Echem (25) reported similar findings for apple juice. According to Iranian National Standard No. 2837, the optimal pH range for non-carbonated fruit beverages is 2.4-4.2 (Iranian National Standard, 2015). The present study demonstrates that all tested beverage treatments-maintained pH levels within this range throughout the analysis period.

3.2. Titratable acidity values

Initially, different beverage treatments exhibited titratable acidity measurements ranging from approximately 0.58-0.61 g/100 mL (Fig. 3). No significant differences were observed between the pasteurized control and epsilon-polylysine treatments ($p>0.05$). During storage, microbial activity, particularly anaerobes, promoted the generation of various organic acids (lactic, succinic, acetic, citric, butyric, and propionic) through fermentation (24). This process contributed to a gradual increase in titratable acidity values over time. On the second day of storage, no significant differences were observed among beverage treatments. By the end of the storage period, the control sample displayed the highest titratable acidity (0.66 g/100 mL), while no statistically significant differences were observed among epsilon-polylysine treatments (0.60-0.62 g/100 mL). Roufegarinejad et al. (21) reported an increase in apple juice titratable acidity during 45-day storage. Nayak et al. (22) and Vollmer et al. (23) noted minimal pasteurization effects on apple and pineapple juice titratable acidity, respectively. Ferreira et al. (24) observed an initial insignificant impact of pasteurization on prickly pear fruit juice titratable acidity, followed by a progressive increase during 40-day storage. Iranian National Standard No. 2837 specifies 0.1-1.7 g/100 mL as the preferred titratable acidity range for non-carbonated fruit beverages (Iranian National Standard, 2015). The present study demonstrates that all investigated beverage treatments consistently complied with this range throughout the analysis period.

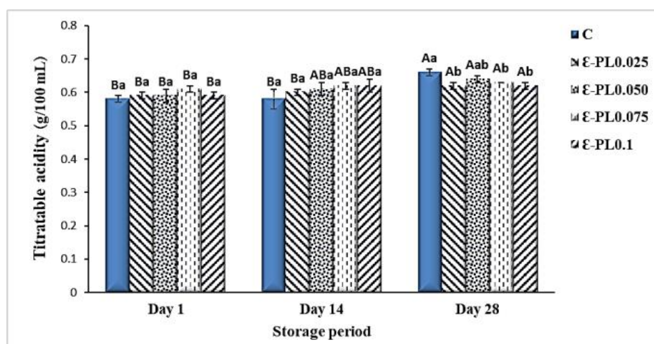

Fig. 3. Changes in titratable acidity values of beverage treatments during the 28-days storage period at 4 °C. Different small and big letters represent significant difference at 5% level of probability among samples and storage period, respectively. C: control (pasteurized sample); Ɛ-PL: unpasteurized sample containing Ɛ-Polylysine.

3.3. Soluble solid content

Soluble solid content, quantified as degrees Brix through refractive index measurement, serves as an indicator of carbohydrate levels (approximately 80%) and includes organic acids, proteins, fats, and minerals present in the sample (26). Analysis of soluble solid content in fruit and vegetable-based beverages during 28-day refrigerated storage (Table 2) revealed that initial values for different beverage treatments ranged from 13.68-13.88°Brix. The pasteurized sample (control) exhibited a statistically significant reduction in soluble solid content compared to treatments with varying concentrations of epsilon-polylysine ($p<0.05$). The decrease in soluble solid content in fruit beverages due to heat pasteurization corresponds with a reduction in reducing sugars, attributed to the initiation of the Maillard reaction at elevated temperatures (24, 27). The Maillard reaction, a chemical pathway contributing to the development of brown-colored compounds in fruit juices during heating and storage, is initiated by the interaction between reducing sugars and amino acids (28). Since the Brix value encompasses both soluble solid content in water and sugars inherent to fruit juices, the reduction in reducing sugars caused by the Maillard reaction, combined with microbial sugar fermentation and conversion to organic acids (e.g., lactic acid) during storage, results in a partial diminution of sugar content, ultimately reducing the Brix value of the beverages (29). Roufegarinejad et al. (21) reported a gradual decline in the soluble solid content of apple juice beverages during 45-day storage, attributed to the metabolic conversion of sugars to organic acids by bacteria (30). Nayak et al. (22) demonstrated minimal impact of pasteurization on apple juice Brix levels, with only a slight reduction observed. Vollmer et al. (23) found that pasteurization induced minimal change in pineapple juice Brix levels. Ayub et al. (31) observed no significant alteration in the soluble solid content of strawberry water over 30-day refrigerated storage. Rabie et al. (32) reported no discernible influence of pasteurization on physalis fruit juice Brix degree, with immaterial changes in soluble solid content during 21-day storage.

Table 2. The total soluble solids, total sugar and formalin number of beverage treatments during 28-days storage period at 4 °C.

Treatments	Time (Day)	Total soluble solids (°Brix)	Total sugar (g/100g)	Formalin number mL/100mL)
	1	13.68 ± 0.07 ^{Ab}	11.89 ± 0.04 ^{Aa}	7.81 ± 0.04 ^{Ab}
C	14	13.65 ± 0.04 ^{Ab}	11.71 ± 0.05 ^{Ba}	7.69 ± 0.06 ^{Bb}
	28	13.60 ± 0.13 ^{Aa}	11.60 ± 0.03 ^{Ca}	7.55 ± 0.03 ^{Cb}
	1	13.85 ± 0.03 ^{Aa}	11.82 ± 0.03 ^{Aa}	8.03 ± 0.06 ^{Aa}
Ɛ-PL0.025	14	13.80 ± 0.04 ^{Aa}	11.70 ± 0.04 ^{Ba}	7.92 ± 0.05 ^{Aa}
	28	13.75 ± 0.07 ^{Aa}	11.63 ± 0.06 ^{Ba}	7.76 ± 0.02 ^{Ba}
	1	13.82 ± 0.05 ^{Aa}	11.88 ± 0.03 ^{Aa}	7.95 ± 0.07 ^{Aa}
Ɛ-PL0.05	14	13.76 ± 0.05 ^{Aa}	11.71 ± 0.07 ^{Ba}	7.87 ± 0.04 ^{Aa}
	28	13.70 ± 0.09 ^{Aa}	11.63 ± 0.02 ^{Ba}	7.69 ± 0.05 ^{Ba}
	1	13.88 ± 0.10 ^{Aa}	11.80 ± 0.05 ^{Aa}	8.03 ± 0.09 ^{Aa}
Ɛ-PL0.075	14	13.82 ± 0.03 ^{Aa}	11.70 ± 0.02 ^{Ba}	7.86 ± 0.08 ^{Aa}
	28	13.81 ± 0.09 ^{Aa}	11.65 ± 0.03 ^{Ba}	7.73 ± 0.06 ^{Ba}
	1	13.85 ± 0.06 ^{Aa}	11.82 ± 0.04 ^{Aa}	7.94 ± 0.08 ^{Aa}
Ɛ-PL0.1	14	13.79 ± 0.05 ^{Aa}	11.72 ± 0.05 ^{ABa}	7.86 ± 0.05 ^{Aa}
	28	13.74 ± 0.06 ^{Aa}	11.65 ± 0.04 ^{Ba}	7.71 ± 0.04 ^{Ba}

Values represent mean (n=3) ± SD. Different small and big letters represent significant difference at 5% level of probability among samples and storage period, respectively. C: control (pasteurized sample); Ɛ-PL: unpasteurized sample containing Ɛ-Polylysine.

Conversely, Jittanit et al. (33) documented a reduction in lemon juice Brix levels following heat pasteurization treatment. Iranian National Standard No. 2837 stipulates a minimum desired level of 10 °Brix for soluble solid content in non-carbonated fruit-based beverages (Iranian National Standard, 2015). This study demonstrates that all examined beverage treatments consistently complied with the soluble solid content range stipulated by the Iranian National Standard throughout the investigation.

3.4. Total sugar content

Analysis of total sugar content in fruit and vegetable-based beverages during 28-day refrigerated storage (Table 2) revealed initial values ranging from 11.11 to 11.89 g/100 g, with no statistically significant differences among treatments ($p>0.05$). A gradual decline in total sugar content was observed throughout storage, attributed to progressive sugar fermentation by microorganisms (34). Sugars serve as substrates metabolized by microorganisms into alcohol or organic acids, with their gradual decline closely linked to consumption by microorganisms inherent in the fruit juice. Vollmer et al. (23) reported similar findings, observing no statistically significant difference in total sugar content between pasteurized and unpasteurized pineapple juice, with pasteurization marginally elevating total sugar content. Emelike and Obinna-Echem (25) demonstrated that heat pasteurization does not substantially impact apple juice sugar content, though a gradual decline in overall sugar content was observed during storage. Conversely, Ferreira et al. (24) reported increased total sugar content in fruit juices post-pasteurization, with Opuntia fruit juices experiencing a decline during 40-day refrigerated storage. Previous studies have also reported elevated total sugar content in apple and sugarcane juices following pasteurization (35, 36).

3.5. Formalin number

The formalin number serves as an indicator of protein and amino acid concentrations in fruit and vegetable-based beverages. This measurement is particularly important as higher amino acid levels can trigger the Maillard reaction when exposed to reducing sugars, leading to darker color in fruit juice (37). Analysis of formalin number during 28-day refrigerated storage (Table 2) revealed initial values ranging from 7.8 to 8.03 mL/100 mL among beverage treatments. The pasteurized sample (control) exhibited a comparatively lower formalin number than treatments with varying epsilon-polylysine concentrations ($p<0.05$). Vollmer et al. (23) observed a reduction in pineapple juice formalin content due to heat pasteurization, aligning with the present study's findings. Throughout refrigerated storage, a consistent decrease in formalin number was observed across beverage treatments, attributed to the Maillard reaction between amino acids and reducing sugars (38). This reaction, involving reducing sugars and amino acids, produces aromatic and brown-colored compounds (39). Given the substantial presence of reducing sugars in fruit and vegetable juices, this reaction progressively unfolds, leading to a gradual decrease in reducing sugars and subsequently initiating a decline in formalin number. Esmaeili et al. (18) reported a gradual and incremental decline in formalin number of blended carrot-orange beverages during 30-day refrigerated storage, aligning with the current study's observations. Iranian National Standard No. 2837 specifies a minimum formalin number of 1.5 mg/100 mL for apple and pear-based fruit beverages (Iranian National Standard, 2015). This study demonstrates that all examined beverage treatments consistently complied with the formaldehyde content range stipulated by the Iranian National Standard throughout the analysis period.

3.6. Viscosity

Analysis of viscosity in fruit and vegetable-derived beverages over a 28-day refrigerated storage period (Fig. 4) revealed initial values ranging from 5.5 to 5.47 centipoise (cP) among different treatments.

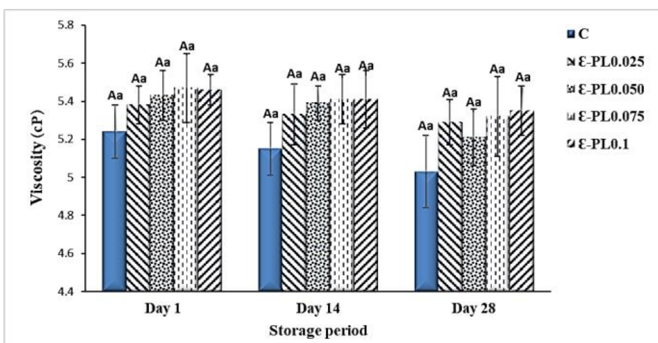

Fig.4. Changes in viscosity values of beverage treatments during the 28-days storage period at 4°C. Different small and big letters represent significant difference at 5% level of probability among samples and storage period, respectively. C: control (pasteurized sample); Ɛ-PL: unpasteurized sample containing Ɛ-Polylysine.

No statistically significant difference was observed between the pasteurized sample (control) and treatments with varying epsilon-polylysine concentrations. Throughout storage, only minor and inconsequential decreases in beverage viscosity were noted ($p>0.05$). Laaksonen et al. (40) reported similar findings, documenting unremarkable alterations in blackberry fruit juice viscosity over 12-month refrigerated storage. Roufegarinejad et al. (21) observed insignificant and subtle viscosity shifts in apple fruit beverages during 45-day storage. Conversely, Jittanit et al. (33) evidenced viscosity reduction in lemon juice due to pasteurization, though no noteworthy alteration was discernible over 4-week refrigerated storage. Emelike and Obinna-Echem (25) substantiated the insignificance of heat pasteurization on apple juice viscosity, citing gradual viscosity decrease during storage, attributed to soluble solids content reduction. In the current study, no substantial reduction in soluble solids content occurred over the storage duration. Consequently, no prominent alteration in beverage consistency was observed, with only minimal and inconsequential reduction in viscosity**.**

3.7. Vitamin C content

Vitamin C (ascorbic acid) is a pivotal parameter for assessing nutritional value and potential health benefits of fruit and vegetable-based beverages. This heat-sensitive nutrient also serves as an indicator of other vitamin degradation (2). Ascorbic acid undergoes reduction in the presence of heat and oxygen (41). Examination of vitamin C content during 28-day refrigerated storage revealed lower levels in the pasteurized sample (control) compared to treatments with varying epsilon-polylysine concentrations ($p<0.05$) (Fig. 5). All beverage treatments exhibited significant decline in vitamin C content during storage ($p<0.05$). Initial vitamin C content ranged from 24.5 to 29.49 mg/100 mL, decreasing to 20.18 to 25.08 mg/100 mL by the end of storage.

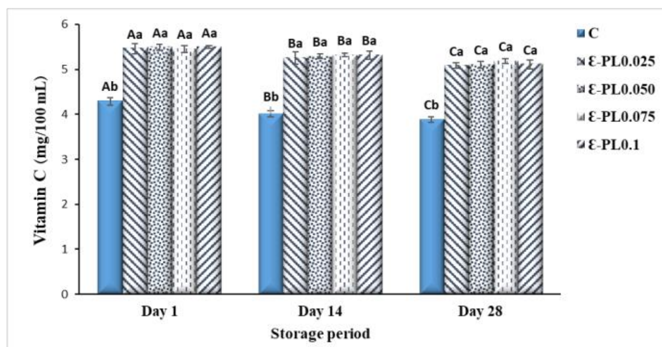

Fig. 5. Changes in vitamin C values of beverage treatments during the 28-days storage period at 4°C. Different small and big letters represent significant difference at 5% level of probability among samples and storage period, respectively. C: control (pasteurized sample); Ɛ-PL: unpasteurized sample containing Ɛ-Polylysine.

Mena et al. (42) documented 40% vitamin C decrease in pomegranate beverages after heat treatment at 65°C for 30 seconds, aligning with the present study. Benjamin and Gamrasni (22) reported substantial vitamin C reduction in pomegranate beverages due to heat pasteurization. Velázquez-Estrada et al. (27) observed decreased vitamin C content in orange juice beverages following heat pasteurization. Wurlitzer et al. (43) noted up to 7% decrease in ascorbic acid content within fruit juices post-pasteurization. L-ascorbic acid transforms into dehydroascorbic acid during heating, a less stable compound that can rapidly and irreversibly degrade into

3,2-diketogluconic acid, resulting in loss of functional attributes (44). The current study observed L-ascorbic acid oxidation during heat pasteurization and storage, leading to vitamin C content reduction in beverage samples. Nayak et al. (22) reported vitamin C reduction in apple juice from 6.24 to 5.08 g/L due to heat pasteurization. Tchuenchieu et al. (2) demonstrated superior vitamin C preservation using a combination of mild heat and carvacrol compared to high-temperature pasteurization. Ayub et al. (31) observed vitamin C content decline in strawberry water during refrigerated storage.

3.8. Color analysis

Color analysis is a crucial quality parameter in the food industry, significantly influencing consumer preferences (45). L*, a*, and b* values correspond to brightness, red-green shift, and blue-yellow shift, respectively (20). Evaluation of color indices over 28-day refrigerated storage (Table 3) revealed initial L*, a*, b*, and ΔE values ranging from 48.31-50.63, 7.22-10.17, 21.23-26.28, and 13.33-15, respectively. The pasteurized sample (control) exhibited lower L* and b* values with higher a* and ΔE values, indicating a darker and redder color profile. No significant differences in color index values were observed among treatments with varying epsilon-polylysine concentrations ($p>0.05$). Pasteurized beverages generally showed more intense reddish color and reduced brightness compared to unpasteurized and epsilon-polylysine-containing counterparts, suggesting accumulation of dark color compounds from heat treatment (32). Throughout storage, significant decreases occurred in L* and b* values, with increased a* and ΔE values ($p<0.05$). Previous research has consistently highlighted thermal pasteurization's deleterious effect on fruit beverage color attributes, corroborated by reduced levels of natural pigments (anthocyanins and carotenoids) post-pasteurization (42, 46).

Table 3. The color indexes of beverage treatments during 28-days storage period at 4 °C.

Treatments	Time (Day)	L*	a*	b*	ΔE
	1	50.63 ± 0.72 Aa	17.22 ± 0.05 Ba	26.41 ± 0.18 Ab	23.33 ± 0.45 Ca
C	14	48.14 ± 0.56 Bb	17.38 ± 0.09 Aa	25.32 ± 0.24 Bb	24.35 ± 0.41 Ba
	28	45.39 ± 0.64 Cb	17.46 ± 0.05 Aa	23.75 ± 0.17 Cb	26.23 ± 0.59 Aa
	1	58.08 ± 0.61 Aa	10.52 ± 0.06 Ab	28.18 ± 0.22 Aa	15.12 ± 0.28 Bb
Ɛ-PL0.025	14	55.95 ± 0.81 Ba	10.59 ± 0.10 Ab	27.63 ± 0.27 Ba	15.41 ± 0.35 Bb
	28	54.11 ± 0.36 Ca	10.65 ± 0.08 Ab	26.51 ± 0.24 Ca	16.10 ± 0.27 Ab
	1	58.31 ± 0.57 Aa	10.46 ± 0.07 Ab	28.02 ± 0.16 Aa	15.10 ± 0.18 Bb
Ɛ-PL0.05	14	55.99 ± 0.62 Ba	10.55 ± 0.07 Ab	26.98 ± 0.33 Ba	15.23 ± 0.34 Bb
	28	53.90 ± 0.68 Ca	10.62 ± 0.11 Ab	26.19 ± 0.30 Ca	16.09 ± 0.46 Ab
	1	58.10 ± 0.59 Aa	10.37 ± 0.11 Ab	28.23 ± 0.14 Aa	15.00 ± 0.31 Bb
Ɛ-PL0.075	14	55.76 ± 0.52 Ba	10.41 ± 0.15 Ab	27.44 ± 0.30 Ba	15.42 ± 0.28 Bb
	28	54.19 ± 0.78 Ca	10.48 ± 0.07 Ab	26.39 ± 0.38 Ca	15.95 ± 0.23 Ab
	1	57.75 ± 0.31 Aa	10.41 ± 0.06 Ab	28.16 ± 0.27 Aa	15.20 ± 0.39 Bb
Ɛ-PL0.1	14	56.42 ± 0.69 Ba	10.47 ± 0.09 Ab	27.11 ± 0.45 Ba	15.38 ± 0.18 Bb
	28	54.09 ± 0.51 Ca	10.54 ± 0.07 Ab	25.99 ± 0.49 Ca	16.05 ± 0.36 Ab

Values represent mean (n=3) ± SD. Different small and big letters represent significant difference at 5% level of probability among samples and storage period, respectively. C: control (pasteurized sample); Ɛ-PL: unpasteurized sample containing Ɛ-Polylysine.

Color alterations during thermal processing primarily stem from natural pigment degradation or Maillard reaction occurrence (47). Tchuenchieu et al. (2) demonstrated limited impact of carvacrol as a natural preservative on orange, pineapple, and watermelon beverage color. Color changes from high-temperature pasteurization were attributed to non-enzymatic browning, pigment degradation, and sugar caramelization. Vollmer et al. (23) documented significant reduction in L*, a*, and b* indices of pineapple water following heat pasteurization. Rabie et al. (32) reported decreased L* and b* indices and elevated a* index in physalis fruit beverages post-pasteurization, with gradual attenuation of brightness, redness, and yellowness during storage. Jittanit et al. (33) observed progressive darkening of lemon water throughout storage.

3.9. Microbiological tests

Heat pasteurization is the predominant method for reducing microbial load and spoilage risk in fruit juices, effectively inactivating heat-sensitive microorganisms including vegetative bacteria, yeasts, and molds (8). Microbiological analysis of fruit and vegetable-based beverages over 28-day refrigerated storage (Table 4) revealed that pasteurized samples (control) and formulations containing 0.075% and 0.1% epsilon-polylysine exhibited total microbial counts and yeast/mold counts below 1 CFU/mL (undetectable) on days 0, 14, and 28. Treatments containing 0.025% and 0.05% epsilon-polylysine showed undetectable microbial counts on days 0 and 14. However, by day 28, these treatments exceeded the permissible thresholds for total microbial count and yeast/mold count, with values less than 1 (No. 3414 Iran National Standard, 2019). The findings align with this standard, with pasteurized samples and treatments containing 0.075% and 0.1% epsilon-polylysine remaining compliant throughout storage. Heat pasteurization disrupts cell membranes and induces structural modifications in nucleic acids and proteins, eliminating microorganisms (22). Epsilon-polylysine, characterized by positively charged amino acid residues, hinders susceptible microorganism proliferation (48) by inducing perturbations in cell wall peptidoglycan layer structure (49) and enhancing membrane permeability through electrostatic interactions (50). Hu et al. (52) demonstrated notable antimicrobial efficacy of epsilon-polylysine in curbing microbial spoilage of apple juice and inhibiting Alicyclobacillus acidoterrestris growth. Lee et al. (53) highlighted substantial antibacterial activity against Gram-positive and Gram-negative bacteria in chicken broth. Li et al. (12) revealed significant mold growth inhibition against Botrytis cinerea in guava juice, elucidating mechanisms including intracellular reactive oxygen species accumulation, downregulation of pathogenicity-linked genes, and precipitation of soluble carbohydrates and nucleic acids. Similar to the present findings, studies by Mandha et al. (54) and Vollmer et al. (23) evidenced that total bacterial count and mold/yeast counts in pasteurized fruit beverages remained below detectable limits (1 log CFU/mL).

Table 4. The microbial load of beverage treatments during 28-days storage period at 4 °C.

Treatments	Time (Day)	Total bacterial count (CFU/g)	Molds and yeasts count (CFU/g)
C	1	<1	<1
	14	<1	<1
	28	<1	<1
Ɛ-PL0.025	1	<1	<1
	14	<1	<1
	28	>1*	>1*
Ɛ-PL0.05	1	<1	<1
	14	<1	<1
	28	>1*	>1*
Ɛ-PL0.075	1	<1	<1
	14	<1	<1
	28	<1	<1
Ɛ-PL0.1	1	<1	<1
	14	<1	<1
	28	<1	<1

Values represent mean (n=3) ± SD. C: control (pasteurized sample); Ɛ-PL: unpasteurized sample containing Ɛ-Polylysine. *: Exceeding national standards.

3.10. Sensory evaluation

The sensory evaluation of fruit and vegetable-based beverages encompassing attributes such as taste, flavor, color, aroma, and overall acceptability was conducted during a 14-day refrigerated storage using a five-point Hedonic test (Table 5). The pasteurized beverage (control) exhibited inferior scores in taste, color, aroma, and overall acceptability compared to the epsilon-polylysine-containing treatments. This decline in sensory scores of the control group can be attributed to the detrimental impact of heat treatment, which unfavorably influences the sensory attributes of the beverages. During heat treatments, flavor and aroma compounds within the fruit juices undergo chemical reactions, including non-enzymatic Maillard reactions and chemical oxidation, resulting in a reduction in the intensity of aromatic compounds in the juices. These chemical reactions also contribute to the deterioration of juice color (20). In contrast, treatments incorporating epsilon-polylysine, owing to the absence of heat treatment, better retained the inherent aroma and flavor compounds as well as the natural color of the fruit and vegetable juices. Over the storage period, a decline in sensory scores was observed, with the control sample and treatments containing 0.025 and 0.05% epsilon-polylysine reaching average overall acceptance scores by the 14th day. In contrast, treatments containing 0.075 and 0.1% epsilon-polylysine sustained favorable overall acceptance scores until the 14th day.

Table 5. The sensory scores of beverage treatments during 14-days storage period at 4 °C.

Treatments	Time (Day)	Taste	Color	Odor	Overall acceptability
C	1	3.50 ± 0.26 Ab	3.20 ± 0.21 Ab	3.50 ± 0.26 Ab	3.30 ± 0.24 Ab
	14	3.00 ± 0.00 Bb	2.70 ± 0.24 Bb	3.00 ± 0.00 Bc	3.00 ± 0.00 Bc
Ɛ-PL0.025	1	4.40 ± 0.24 Aa	4.30 ± 0.24 Aa	4.60 ± 0.26 Aa	4.60 ± 0.26 Aa
	14	4.00 ± 0.00 Ba	4.00 ± 0.00 Ba	3.50 ± 0.26 Ba	3.50 ± 0.26 Bb
Ɛ-PL0.05	1	4.20 ± 0.21 Aa	4.30 ± 0.24 Aa	4.40 ± 0.26 Aa	4.40 ± 0.26 Aa
	14	4.00 ± 0.00 Aa	4.00 ± 0.00 Ba	3.80 ± 0.24 Bb	3.60 ± 0.26 Bb
Ɛ-PL0.075	1	4.20 ± 0.21 Aa	4.30 ± 0.24 Aa	4.30 ± 0.24 Aa	4.40 ± 0.26 Aa
	14	4.10 ± 0.16 Aa	4.10 ± 0.16 Aa	4.00 ± 0.00 Ba	4.10 ± 0.16 Aa
Ɛ-PL0.1	1	4.10 ± 0.16 Aa	4.40 ± 0.26 Aa	4.40 ± 0.26 Aa	4.40 ± 0.26 Aa
	14	4.00 ± 0.00 Aa	4.00 ± 0.00 Ba	4.00 ± 0.00 Ba	4.00 ± 0.00 Aa

Values represent mean (n=3) ± SD. Different small and big letters represent significant difference at 5% level of probability among samples and storage period, respectively. C: control (pasteurized sample); Ɛ-PL: unpasteurized sample containing Ɛ-Polylysine.

Consistent with the present study, Benjamin and Gamrasni (20) emphasized that the utilization of heat treatment in pasteurization, due to elevated temperatures, exerts a negative influence on the sensory properties of fruit juices. Nayak et al. (22) similarly observed a reduction in sensory scores for taste, aroma, color, and overall acceptance of apple juice owing to pasteurization. However, the sensory scores of pasteurized beverages remained within acceptable ranges. Contrarily, Wurlitzer et al. (43) noted that in pasteurized fruit-flavored beverages, sensory acceptance endured well over a 180-day storage period, with minimal alterations in overall acceptance during this duration. Likewise, Rabie et al. (32) reported that despite slight declines in sensory scores for attributes like color, aroma, and flavor, taste, mouthfeel, and overall acceptance, both pasteurized and unpasteurized physalis fruit juice maintained high sensory acceptance levels for up to 21 days of refrigerated storage.

4. Conclusion

The findings of this study indicate notable changes in various parameters of fruit and vegetable-based beverages during their storage period. The pH, total sugar content, formalin number, vitamin C, and color indices L* and b* exhibited a declining trend, while titratable acidity, redness, and overall color changes displayed an increasing pattern. Comparatively, the pasteurized sample (control) demonstrated diminished brightness and yellowness coupled with heightened redness when contrasted with unpasteurized and epsilon-polylysine-treated beverage variants, also manifesting more pronounced overall color changes. The vitamin C and soluble solids content, along with formalin number, were significantly lower in the pasteurized sample compared to unpasteurized and epsilon-polylysine-treated counterparts. Heat pasteurization treatment had minimal impact on beverage viscosity. Microbiologically, the pasteurized sample and higher epsilon-polylysine concentrations (0.075 and 0.1%) remained microorganism-free throughout the storage period (28th day), whereas lower epsilon-polylysine levels (0.025 and 0.05%) exhibited microbial presence by the study's end. Sensory evaluation echoed the substantial influence of substituting heat pasteurization with epsilon-polylysine treatment, emphasizing the preservation of sensory acceptance and organoleptic characteristics. The pasteurized sample scored lowest in taste, color, aroma, and overall acceptance, while maintaining moderate sensory scores. Overall, these outcomes highlight the potential for producing high-quality, safe beverages through epsilon-polylysine utilization instead of heat pasteurization. Notably, treatments incorporating 0.075 and 0.1% epsilon-polylysine exhibited the most favorable sensory attributes.

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