applied microbiology

MDPI

Article

Effects of Vacuum Pasteurization on the Nutritional, Sensory and Microbiological Properties of Orange (*Citrus* × *sinensis*) and Carrot (*Daucus carota* L.) Nectar

Llerena-Silva Wilma [1], José Burgos [2], Jacqueline Ortiz [2], Iván Samaniego [3,4], Jhunior Marcia [5], Molina José [1], Christian Vallejo [1], Ignacio Angós [2,6], Ajitesh Yaday [7] and Ricardo Santos Alemán [8,*]

1 Faculty of Industry and Production Sciences, Food Engineering, State Technical University of Quevedo (UTEQ), Km 7 1/2 via Quevedo-El Empalme, Quevedo 120313, Ecuador; wllerenas@uteq.edu.ec (L.-S.W.); jose.molina2016@uteq.edu.ec (M.J.); cvallejo@uteq.edu.ec (C.V.)
2 Faculty of Science and Engineering in Food and Biotechnology, Technical University of Ambato (UTA), Av. Los Chasquis and Río Payamino, Ambato 180103, Ecuador; jburgos4775@uta.edu.ec (J.B.); jortizes@uta.edu.ec (J.O.); ignacio.angos@unavarra.es (I.A.)
3 Department of Nutrition and Quality, Santa Catalina Research Site, National Institute of Agricultural Research (INIAP), Cutuglahua 171107, Ecuador; ivan.samaniego@iniap.gob.ec
4 Posgrado Desarrollo e Innovación de Alimentos, Universidad de las Américas (UDLA), Quito 170503, Ecuador
5 Facultad de Ciencias Tecnológicas, Universidad Nacional de Agricultura, Catacamas 16201, Honduras; jmarcia@unag.edu.hn
6 Department of Agronomy, Biotechnology and Food, Public University of Navarra (UPNA), Campus Arrosadia, 31006 Pamplona, Spain
7 Department of Food Science and Human Nutrition, Iowa State University, Ames Iowa, IA 50011, USA; ajiteshyadav10603@gmail.com
8 School of Nutrition and Food Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA 70802, USA
* Correspondence: rsantosaleman@lsu.edu; Tel.: +1-225-846-2540

Abstract: This study involved the evaluation of the effect of vacuum pasteurization on physico-chemical characteristics (pH, total soluble solids, titratable acidity, chroma, tone, IO, vitamin C, 5-hydroxymethylfurfural), microbiological properties (*Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, total coliforms, total mesophilic aerobes, molds and yeasts) and sensory characteristics of orange and carrot nectar. The thermal treatments were designed based on the thermal lethality of two heat-resistant microorganisms typical of the product (*Neosartorya fischeri* and *Zygosaccaromyces bailii*). The evaluation was carried out on raw nectar and pasteurized nectar. The shelf life was estimated to be 30 days (6 °C). The most favorable results were obtained by applying a heat treatment at 88 °C for 32.68 min, managing to retain 85.87% of vitamin C and a microbiological stability of 12 days (6 ± 0.6 °C) with regard to total mesophilic aerobes. Likewise, the tasters established that this treatment resulted in the best flavor, texture and acceptability characteristics.

Keywords: vacuum pasteurization; thermal processing; shelf life; fruit and vegetable beverages; HTST pasteurization; LTLT pasteurization

Citation: Wilma, L.-S.; Burgos, J.; Ortiz, J.; Samaniego, I.; Marcia, J.; José, M.; Vallejo, C.; Angós, I.; Yaday, A.; Alemán, R.S. Effects of Vacuum Pasteurization on the Nutritional, Sensory and Microbiological Properties of Orange (*Citrus* × *sinensis*) and Carrot (*Daucus carota* L.) Nectar. *Appl. Microbiol.* **2024**, *4*, 731–744. https://doi.org/10.3390/applmicrobiol4020050

Academic Editor: Filipa Silva

Received: 19 February 2024
Revised: 22 April 2024
Accepted: 23 April 2024
Published: 28 April 2024

1. Introduction

Pasteurization is a process to which certain liquids, such as beverages, are subjected to eliminate pathogens that could make people sick when consumed. Thanks to its use, infections and food poisoning are becoming less frequent. Pasteurization must be carried out strictly following the recommended time–temperature relationship since the process can be very dangerous should any pathogen survive. On the other hand, pasteurization at a temperature higher than the recommended one leads to a reduction in the nutritional value of beverages, evidenced by the loss of vitamins (such as riboflavin, ascorbic acid,

and others) and also a reduction in the availability of some essential amino acids such as lysine, combined with the negative effect on the organoleptic characteristics of the product obtained. In pasteurization, bacteria such *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Coxiella burneti* are eliminated. Fruits and vegetables are nutritional sources [1]. Orange (*Citrus* × *sinensis*) has high vitamin C, fiber, organic acid (citric acid), carbohydrate and phytonutrient content [2]. Carrot is a vegetable with a high concentration of carotenoids, vitamins (B_1, B_2, B_3, B_6, B_7, C, E and K), sugars and minerals. These nutrients are necessary for the human body to carry out its biological functions [1]. They can act as antioxidants and can have antimicrobial, antiviral, anti-inflammatory, antiallergenic and anticancer effects [3]. Juice and nectar are different products. Juice is 100% pure fruit juice, or close to it, and nectar is a drink made from fruit juice or pulp, water and sugar. Nectars can contain many of the nutrients of the fruits and vegetables with which they were made. However, they are susceptible to nutritional and organoleptic deterioration because of thermal processing. The growth of microorganisms in food may be associated with the natural microbiota of the raw material and unhygienic processing conditions [4].

The loss of color and the formation of precipitates in nectar are quality alterations due to the enzymatic activity of polyphenol oxidases and pectin methylesterases [5,6]. In acidic products, the growth of yeasts (*Kloeckera*, *Candida*, *Cryptococcus*, *Debaryomyces*, *Saccharomyces*, *Zygosaccharomyces*, *Pichia* and *Rhodotorula*) and molds (*Penicillium*, *Neosartorya*, *Byssochlamys*, *Talaromyces* and *Eupeniccilium*) is evident. In orange and carrot nectar, *Neosartorya fischerii* and *Zygosaccharomyces bailii* are two heat-resistant microorganisms of interest [7–10].

Neosartorya fischerii is a mold that produces pectinolytic enzymes that can alter nectar quality. The mycotoxins (fumitremorgens A, B, and C and verruculogen) generated by this microorganism can interact with the central nervous system, causing tremors, convulsions and death [11]. *Zygosaccharomyces bailii* is a yeast responsible for the rapid fermentation of sugars in fruit nectar [12]. Thermal pasteurization is a process applied to reduce the microbial load of a food product to a desired level and inactivate enzymes that could alter it. Different types of pasteurization can be applied, such as slow pasteurization (69 °C for 30 min), high-temperature pasteurization and short times (72–100 °C for 15–0.01 s) and ultra-pasteurization (138 °C for 2 s). During this process, it is necessary to control the times and temperatures applied to reduce thermal damage to the product, such as the loss of vitamin C and the modification of sensory and physicochemical properties [13–15].

Vacuum thermal pasteurization is a thermal treatment that reduces the boiling point of liquid substances by eliminating air inside the cooking pot. This process slows down oxidation reactions in the product. By reducing the pressure to which the liquid particles are subjected, the physicochemical and sensory properties of the nectar are preserved [16]. The objective of the present investigation was to study the influence of vacuum pasteurization on the nutritional, sensory and microbiological properties of orange and carrot nectar, for which two lethality models were proposed.

2. Materials and Methods

2.1. Orange and Carrot Nectar Preparation

To prepare the nectar, fruit previously disinfected with a sodium hypochlorite solution (4.5%; *w*/*v*; Clorox, Guayaquil, Ecuador) was used. A Skymsem semi-automatic juicer (Siemsen, Itaim Bibi, Brazil) was used to extract orange juice. A Mega Mouth Juicer BM5330 vegetable extractor (Omega Juicers, Pennsylvania, PA, USA) was used for carrot juice. The formulation was made with orange juice, carrot juice and natural still mineral water (CBC-Tesalia, Thessaly, Ecuador) in proportion (1:1:2; *v*/*v*/*v*). The soluble solid concentration of the resulting mixture was adjusted to a range of 14 to 16 °Brix (Pocket-Atago brixometer; Tokyo, Japan) with food-grade sucrose (Azucar Valdez, Valdez, Ecuador). The pH of the nectar was standardized in a range between 4.3 and 4.7 according to what was established [17]. The thermal treatment of nectar was proposed based on the thermal lethality of two target microorganisms: *Neosartorya fischerii* (Z = 4; D 88 °C (min) = 1.2–7.5 min) [11] and *Zygosaccharomyces bailii* (Z = 7.19; D 60 °C = 4 s) [12]. Thermal processing was carried

out using a LAB50 multipurpose module (DeLorenzo, Rozzano, Italy). The temperature checks in the thermal processing were carried out with the help of E-val flex wired thermorecorders (Ellab A/S; Hillerød, Denmark) controlled by Valsuite Pro software (Ellab A/S; Hillerød, Denmark) [18]. At the end of the pasteurization process, the nectar was packaged in 280 mL glass bottles (the bottles were sterile and filled aseptically), and rapid cooling in ice water was applied. The samples were stored at 6 ± 0.6 °C (HACEB RVC-17 EXP refrigerator, 402 L; Bogotá, Colombia). Three replications were carried out in all experiments.

2.2. Physicochemical Analysis

The physicochemical analysis of the nectar samples was carried out based on parameters such as pH [19], titratable acidity [20] and soluble solids [21]. The determinations were carried out in triplicate. For color analysis, it was carried out with a Hunter Lab colorimeter to obtain the L*, a* and b* values from the International Commission on Illumination (CIELab) and thus interpret them with the help of equations and the graphic system [22]. The results are described in Table 1.

Table 1. Color and physicochemical changes in orange and carrot nectar using 6 thermal treatments: lightness (L*), a*, b*, chroma (C*), hue (°h), darkening index (DI), percentage of citric acid (%), total soluble solids (°Brix), pH and 5-HMF.

Parameters	Sample	Thermal Treatments											
		T1 (92 °C/3.3 min)		T2 (90 °C/10.3 min)		T3 (88 °C/32.7 min)		T4 (70 °C/2.3 min)		T5 (65 °C/11.4 min)		T6 (60 °C/56.6 min)	
L*	Raw nectar	33.41 ± 1.13	Bd	52.42 ± 11.16	Abc	57.76 ± 3.99	Aab	48.60 ± 12.66	Aa	33.87 ± 4.66	Ad	47.7 ± 10.59	Acd
	Pasteurized	42.05 ± 2.55	Ac	61.07 ± 4.36	Aab	61.85 ± 19.37	Aa	65.60 ± 10.22	Aa	34.02 ± 7.62	Ac	43.89 ± 3.80	Abc
a*	Raw nectar	10.47 ± 0.46	Ba	7.91 ± 1.42	Ab	6.41 ± 0.85	Abc	7.77 ± 1.86	Ac	8.72 ± 0.45	Ab	7.54 ± 1.38	Ab
	Pasteurized	9.65 ± 0.03	Aa	5.97 ± 0.46	Ab	6.23 ± 3.04	Ab	5.23 ± 1.96	Ab	7.68 ± 0.14	Aab	7.26 ± 0.51	Aab
b*	Raw nectar	18.23 ± 0.43	Ba	18.29 ± 1.26	Aa	16.71 ± 1.85	Aa	18.02 ± 1.24	Aa	16.43 ± 1.56	Aa	16.30 ± 1.35	Aa
	Pasteurized	23.29 ± 0.02	Aa	17.71 ± 0.34	Ab	17.34 ± 5.22	Ab	16.12 ± 2.79	Ab	16.61 ± 0.64	Ab	17.55 ± 0.52	Ab
Chroma (C*)	Raw nectar	21.02 ± 0.59	Ba	19.94 ± 1.57	Aab	17.90 ± 2.01	Ab	19.65 ± 1.84	Ab	18.61 ± 1.58	Aab	17.97 ± 1.79	Aab
	Pasteurized	25.21 ± 0.01	Aa	18.73 ± 0.12	Ab	18.45 ± 5.92	Ab	16.97 ± 3.23	Ab	18.30 ± 0.53	Ab	18.99 ± 0.60	Ab
Hue (°h)	Raw nectar	12.29 ± 0.28	Bc	16.34 ± 2.18	Bbc	18.11 ± 0.80	Aab	16.64 ± 3.19	Aa	13.24 ± 0.62	Abc	15.31 ± 1.69	Ab
	Pasteurized	16.82 ± 0.07	Abc	20.76 ± 1.13	Aab	20.70 ± 4.92	Aab	22.25 ± 5.35	Aa	15.14 ± 0.79	Ac	16.86 ± 0.99	Aabc
Dark index (DI)	Raw nectar	82.88 ± 5.01	Ba	45.83 ± 17.38	Acd	33.64 ± 6.23	Acd	50.51 ± 20.16	Ad	64.43 ± 4.55	Aab	46.17 ± 15.28	Abc
	Pasteurized	71.77 ± 0.67	Aa	33.50 ± 5.42	Ab	37.25 ± 24.30	Ab	27.49 ± 10.38	Ab	68.78 ± 16.46	Aa	50.27 ± 6.95	Aab
Total soluble solids (°Brix)	Raw nectar	14.71 ± 0.12	Aab	15.68 ± 0.84	Aa	14.05 ± 1.26	Ab	15.12 ± 1.23	Aab	15.06 + 0.42	Aab	14.71 ± 0.30	Aab
	Pasteurized	16.67 ± 1.49	Aa	15.96 ± 1.40	Aa	14.72 ± 0.17	Aa	16.41 ± 2.43	Aa	14.71 ± 0.55	Aa	14.67 ± 0.78	Aa
Citric acid (%)	Raw nectar	0.23 ± 0.04	Ad	0.14 ± 0.01	Aa	0.18 ± 0.01	Ab	0.22 ± 0.01	Acd	0.19 ± 0.01	Abc	0.23 ± 0.01	Ad
	Pasteurized	0.24 ± 0.01	Ad	0.14 ± 0.01	Aa	0.18 ± 0.01	Ab	0.20 ± 0.02	Abc	0.19 ± 0.01	Ab	0.22 ± 0.02	Acd
pH	Raw nectar	4.09 ± 0.10	Ac	4.33 ± 0.03	Ab	4.45 ± 0.04	Aa	4.32 ± 0.03	Ab	4.37 ± 0.02	Aab	4.39 ± 0.01	Aab
	Pasteurized	4.21 ± 0.08	Ac	4.33 ± 0.03	Ab	4.45 ± 0.04	Aa	4.34 ± 0.03	Ab	4.42 ± 0.03	Aa	4.40 ± 0.001	Aab
5-HMF	Raw nectar	*ND		1.37 ± 0.15	A	*ND		*ND		*ND		*ND	
	Pasteurized	1.05 ± 0.05	a	1.30 ± 0.20	Aa	1.45 ± 0.45	a	*ND		*ND		*ND	

A, B: Significant differences ($p < 0.05$) between orange and carrot nectar raw and pasteurized nectar. a, b, c, d: Significant differences ($p < 0.05$) between thermal treatments (T1, T2, T3, T4. T5, T6). *ND: Not detected.

2.3. Vitamin C

For the quantification of vitamin C, a semiquantitative method was proposed by [23]. The determinations were carried out in duplicate using strips impregnated with molybdophosphoric acid. Ascorbic acid reduced yellow molybdophosphoric acid to phosphor molybdenum blue, which was determined by spectrophotometry on the RQflex® apparatus (Sigma Aldrich, St Louis, MO, USA).

2.4. Hydroxymethylfurfural

The reflectometric method was used to determine the concentration of 5-Hydroxymethylfurfural [24]. The determinations were duplicated using strips impregnated with a barbituric acid derivative and an aminophenazone derivative. The result was expressed in $mg \cdot L^{-1}$.

2.5. Microbiologic Analysis

To determine the content of total mesophilic aerobes (AMTs) (INEN 1529-5) [25] and molds—yeasts (ML) (INEN 1529-10 [26]), microbiological seedings were carried out on plate count agar (PCA) and potato dextrose agar (PDA), respectively. Nectar samples were diluted (10-1, 10-2) in peptone water. Incubation was carried out at 37 ± 1 °C for 48 h for AMT and 25 ± 1 °C for 3–5 days.

The presence of pathogenic microorganisms was determined through Petrifilm plates [27] for *Staphylococcus aureus* (Petrifilm Staph Express plates, [28]), *Listeria monocytogenes* (Petrifilm plates for monitoring *Listeria* in environments, [29]), total coliforms and *Escherichia coli* (Petrifilm *E. coli*/coliforms plates) [30]. Incubation was carried out at 35 °C for 24 h for the first three cases and 48 h for *Escherichia coli*. The results are described in Table 2; there was no detection of pathogenic bacteria.

Table 2. Effectiveness of the pasteurization process in inhibiting *Listeria monocytogenes* in 6 treatments of orange and carrot nectar, treated by vacuum cooking.

Thermal Treatments	Inhibiting *Listeria monocytogenes*	
	Raw Nectar	Pasteurized
T1 (92 °C/3.3min)	Absence	Absence
T2 (90 °C/10.3min)	Absence	Absence
T3 (88 °C/32.7min)	Absence	Absence
T4 (70 °C/2.3min)	Absence	Absence
T5 (65 °C/11.4min)	Absence	Absence
T6 (60 °C/56.6 min)	Absence	Absence

NEN standard limit: Not detected in 25 g.

2.6. Sensory Analysis

The sensory analysis of the nectar samples was carried out with a panel of 77 untrained tasters based on hedonic and descriptive parameters with a complete block design. The liking scores were obtained with a 9-point hedonic test to describe the variables of color, aroma, flavor, sweetness and acidity. Each consumer was given the 3 formulations and the control in an order of service established by preliminary permutations following the methodology proposed by Alemán et al. (2024) [22], with slight modifications.

2.7. Statistical Analysis

Statistical analyses were carried out by the Statgraphics Centurion XVII statistical package (Statpoint Technologies Inc.; Virginia, USA), with a blocking factor design with several levels equal to the number of treatments, using a level of 95% significance ($\alpha = 0.05$); the results were analyzed using analysis of variance (ANOVA). Tukey's Honest Significant Difference (HSD) test was applied to the samples with significant differences.

3. Results and Discussion

3.1. pH

Figure 1 shows that when applying the different thermal treatments, the pH of the nectar tended to increase; in raw samples, the pH was between 4.09 and 4.39. The pasteurized samples had a pH of 4.21 to 4.45 (Table 1). The increase in pH in a pasteurized product was due to a dissociation of acids in the water due to heat treatment. However, no statistically significant differences were found between treatments ($p = 0.0535$), which agrees with the study presented by Santhirasegaram et al., 2015, which mentions that pasteurizing mango juice at 90 °C for one minute did not present significant differences in this parameter. During cold storage, the pH was maintained for up to 30 days (6 ± 0.6 °C) [31,32].

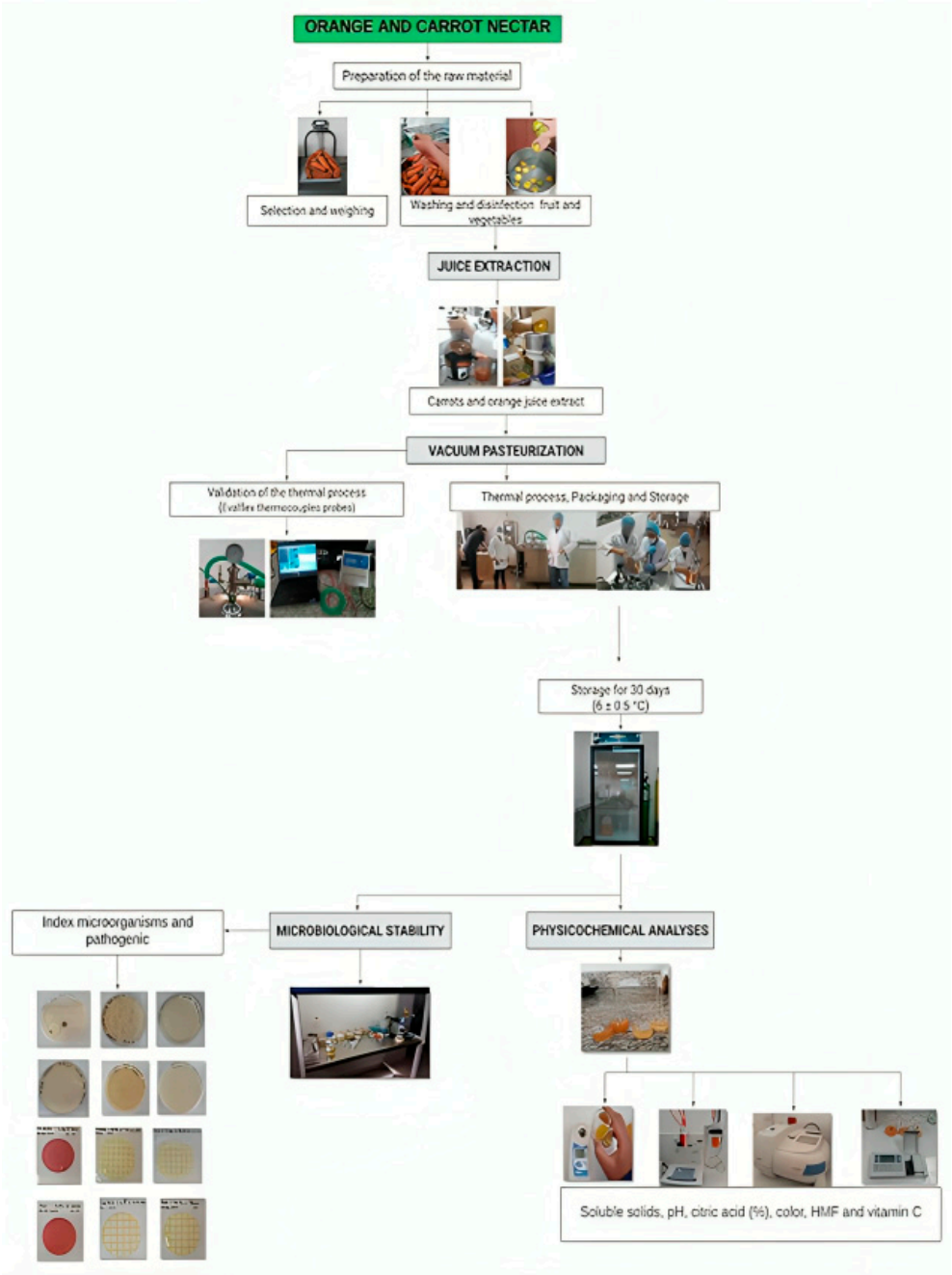

Figure 1. Flow diagram of the vacuum pasteurization process for orange and carrot nectar.

3.2. Titratable Acidity

According to Rivas et al. (2006) [14], citric acid is the predominant organic acid in orange and carrot nectar. Figure 1 shows that when applying the heat treatment, a slight decrease in organic acids was observed in the raw samples. However, treatment 6 (T6: 92 °C for 3.3 min) presented a slight increase in these parameters (Table 1). These results disagree with the studies presented by Igual et al. (2010) [33] and Rivas et al. (2006) [14], who established no significant differences between the pasteurized and raw samples. During cold storage, no variation was found in the percentage of citric acid in the samples.

3.3. Total Soluble Solids

Figure 1 shows that the concentration of total soluble solids in the nectar increased after pasteurization. The variation between treatments was not statistically significant. The increase may have been due to water loss due to evaporation in thermal treatments. The

increase in TSS can occur during storage due to the formation of soluble products due to the degradation of sugars [5]. During storage (30 days), there was no variation in this parameter; however, the prolonged storage of products can have an impact on TSS [14].

3.4. Color

Hue is a variable angular measurement between 0 and 360° of the hue angle of the positive axis of a* that corresponds to the visual sensation of similarity that an area reflects towards a color or combination of several [34]. According to Figure 2 and Table 1, the tone of the samples did not present significant differences (p = 0.1461) after pasteurization, which confirms the results obtained by Rivas et al. (2006) [14], who established that after pasteurization (98 °C for 21 s), the orange and carrot juice did not present statistically significant differences in tone. During storage (30 days at 6 °C), a decrease in tone was observed; it presented a greater variation in the thermal treatments pasteurized at 92 °C for 3.3 min and 90 °C for 10.30 min. According to Min et al. (2003) [35] and Choi et al. (2002) [36], the loss of tone during storage may be due to non-enzymatic reactions and the loss of chemical compounds such as vitamin C and carotenoids.

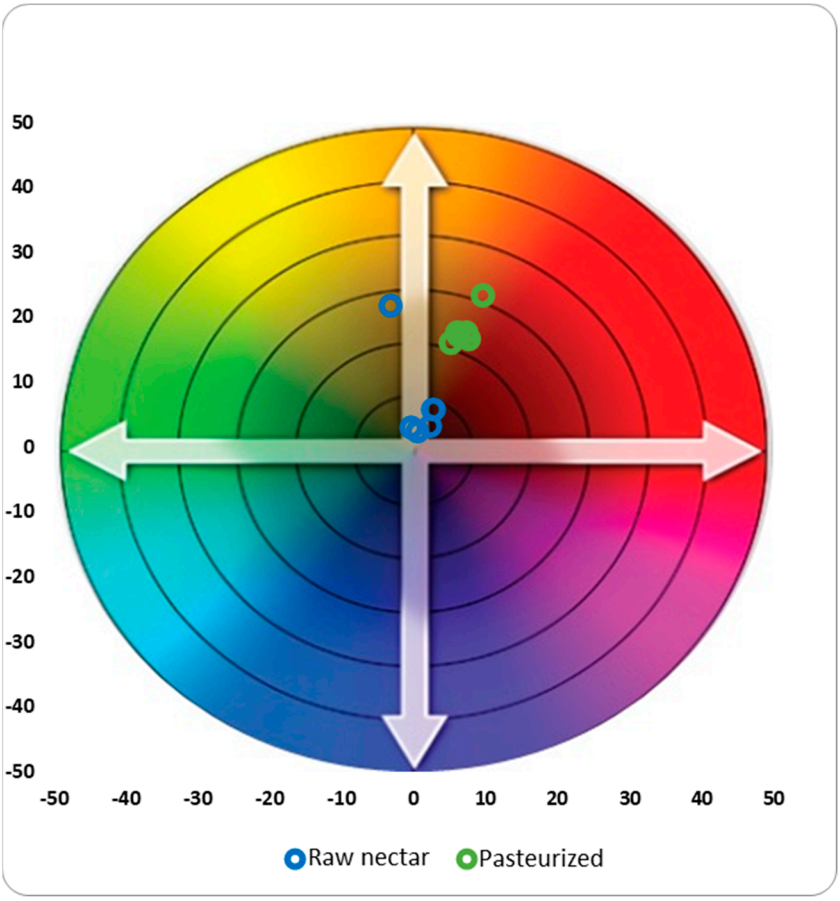

Figure 2. Color changes in orange and carrot nectar using 6 heat treatments. Raw nectar (o), pasteurized nectar (o).

In chroma, a significant effect of vacuum pasteurization was not evident in the raw samples, increasing in those treatments at high temperatures or long application times. On the contrary, treatments T2, T4 and T5 showed a decrease in this parameter. When applying a heat treatment to a food product, an increase in chroma is evident due to the presence of products of the Maillard reaction and enzymatic darkening due to the effect of polyphenol oxidase (PPO) [37,38].

Likewise, during storage, the chroma decreased; however, the change was greater in treatments T4, T5 and T6 because the temperatures and times applied did not achieve the inactivation of the PPO [36,39]. Similarly, it is observed in Figure 1 that the darkening index was influenced by the time (p = 0.2286) of the thermal treatments, being lower in treatments T1, T2 and T4, whose pasteurization time was short, while prolonged heat treatments caused an increase in dark index (DI) (T3, T5 and T6). These results confirmed the study presented by Cortes et al. (2008) [39], who observed an increase in DI in orange juice pasteurized at 90 °C for 20 s. During storage (6 °C for 30 days), the DI did not show a particular trend; these results confirm the work presented by Choi et al. (2002) [36], who stated that the dark index increased and decreased when juice was stored at 4.5 °C for 7 weeks.

3.5. Vitamin C

In Figure 3, the vitamin C content of the raw samples decreased after heat treatment; nectars subjected to high pasteurization temperatures in T1, T2 and T3 presented a higher percentage of vitamin C loss. Vitamin C is sensitive to heat and oxygen, showing loss of this component when vacuum pasteurization is applied to nectars treated at 65 °C for 12 and 7 min, respectively. During storage, vitamin C can be degraded by oxidation and the presence of light, temperature and storage time. The highest vitamin C loss occurred during storage (6 °C for 30 days) in orange and carrot nectar [40]. Prolonged exposure to oxidation accounted for the destruction of a considerable amount of vitamin C by slow pasteurization. Other processes, such as pressing, resulted in a loss of about 22% of vitamin C. Pasteurization at a high temperature (85 °C) reduced the ascorbic acid content by 35% compared to filtered juice [40].

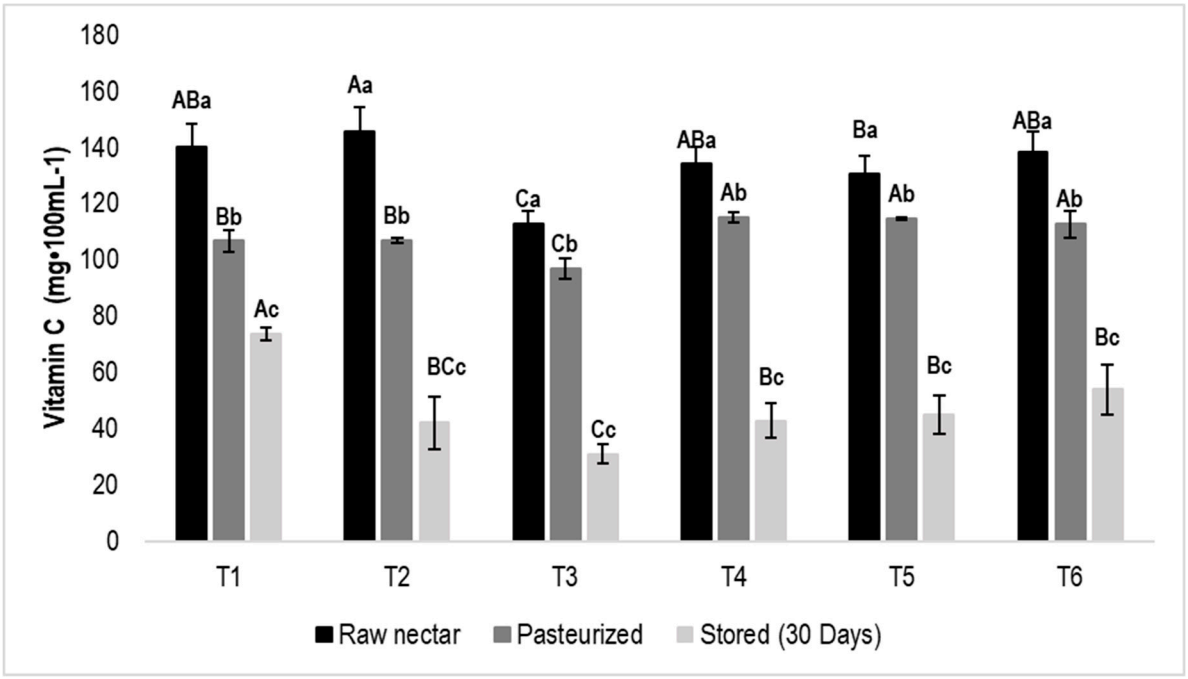

Figure 3. Degradation of vitamin C in orange and carrot nectar using 6 thermal treatments: T1: 92 °C/3.3 min; T2: 90 °C/10.3min; T3: 88 °C/32.7min; T4: 70 °C/2.3min; T5: 65 °C/11.4min; T6: 60 °C/56.6 min. Raw nectar (■), pasteurized (■) and stored at 6 °C for 30 days (■). A, B, C: Significant differences ($p < 0.05$) between thermal treatments (T1, T2, T3, T4, T5, T6). a, b, c: Significant differences ($p < 0.05$) between orange and carrot nectar raw and pasteurized nectar.

3.6. Determination of 5-Hydroxymethylfurfural

In the different treatments of orange and carrot nectar, 5-HMF was not detected (Table 1), which allowed us to establish that the intensity of the thermal treatments applied allowed the maintenance of the quality of the product without causing the thermal degradation of sugars such as fructose, sucrose and glucose [13].

3.7. Microbiological and Sensory Properties

Figure 4 shows that treatments T3 (16 days; 178 CFU·mL^{-1}) and T4 (19 days; 373 CFU·mL^{-1}) presented greater stability concerning the development of total mesophilic aerobes than treatments T1 (19 days; 117 CFU·mL^{-1}), T2 (9 days; 17 CFU·mL^{-1}), T5 (5 days; 13 CFU·mL^{-1}) and T6 (5 days; 50 CFU·mL^{-1}) (INEN (the National Standards Body of the Republic of Ecuador) 2008); this can be attributed to the quality of the material used for the formulations, since in the raw samples, an excessive microbial load was found. These microorganisms are indicators of poor hygiene during food processing [41]. However, after each heat treatment was applied, the AMT amount was reduced below the limits permissible by the INEN 2337 standard [25,26]. T1 was more effective than T4 against the development of AMT for 19 days below acceptable limits.

Figure 4 shows that the development of yeasts in the raw samples exceeded the permissible limit in the INEN 2337 standard for treatments T2 (38 CFU·mL^{-1}), T5 (504 CFU·mL^{-1}) and T6 (175 CFU·mL^{-1}); this may be due to excessive initial contamination of the raw material (T1 93 CFU·mL^{-1}; 30 days) (T3 262 CFU·mL^{-1}) (T4 50 CFU·mL^{-1}; 5 days) and low pasteurization temperatures (T5 65 °C and T6 60 °C) (Batt and Tortorello, 2014).

During storage (6 °C for 30 days), all thermal treatments managed to avoid the development of molds within the limit established by the INEN 2337 standard except T6 (30 days; 80 CFU·mL^{-1}). Treatments T1, T2, T3, T4 and T5 (30 days; <10 CFU·mL^{-1}) slowed down yeast growth for a longer period (23 days).

The growth of pathogenic microorganisms such as *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* was not evident in any treatments evaluated. However, the raw samples showed high microbial contamination by total coliforms. After applying vacuum pasteurization, the pathogenic microbial load was reduced below the permissible limits of the INEN 2337 standard in all treatments (Figure 5). T5 and T6 showed counts below the INEN standards for mesophilic aerobic bacteria, *Enterobacteriaceae*, molds and *Escherichia coli*, whereas T6 had higher counts for yeast and *Staphylococcus aureus*. Not surprisingly, yeast and *Staphylococcus aureus* were more heat resistant than mesophilic aerobic bacteria, *Enterobacteriaceae*, molds and *Escherichia coli.*

Figures 6 and 7 show that in acceptability (hedonic parameters), the treatments that were pasteurized at temperatures below 70 °C (T4, T5 and T6) preserved the color of the nectar, presenting a higher score than treatments T1, T2 and T3. However, in terms of smell, flavor, texture and acceptability, the tasters did not detect significant differences between the treatments. According to Figure 5, the intensity of the attributes (descriptive parameters), such as color, was greater in treatments T1 and T2. According to the panel of tasters, this was darker than in treatments T3, T4, T5 and T6. The smell was more intense in short thermal treatments such as T1 and T4, which better preserved the aroma of the nectar. Sweetness and acidity did not present significant differences (p = 0.7220; p = 0.3391) between the treatments and the control. In texture, the panel of evaluators determined that the treatments subjected to pasteurization were more viscous than the control treatment (raw nectar).

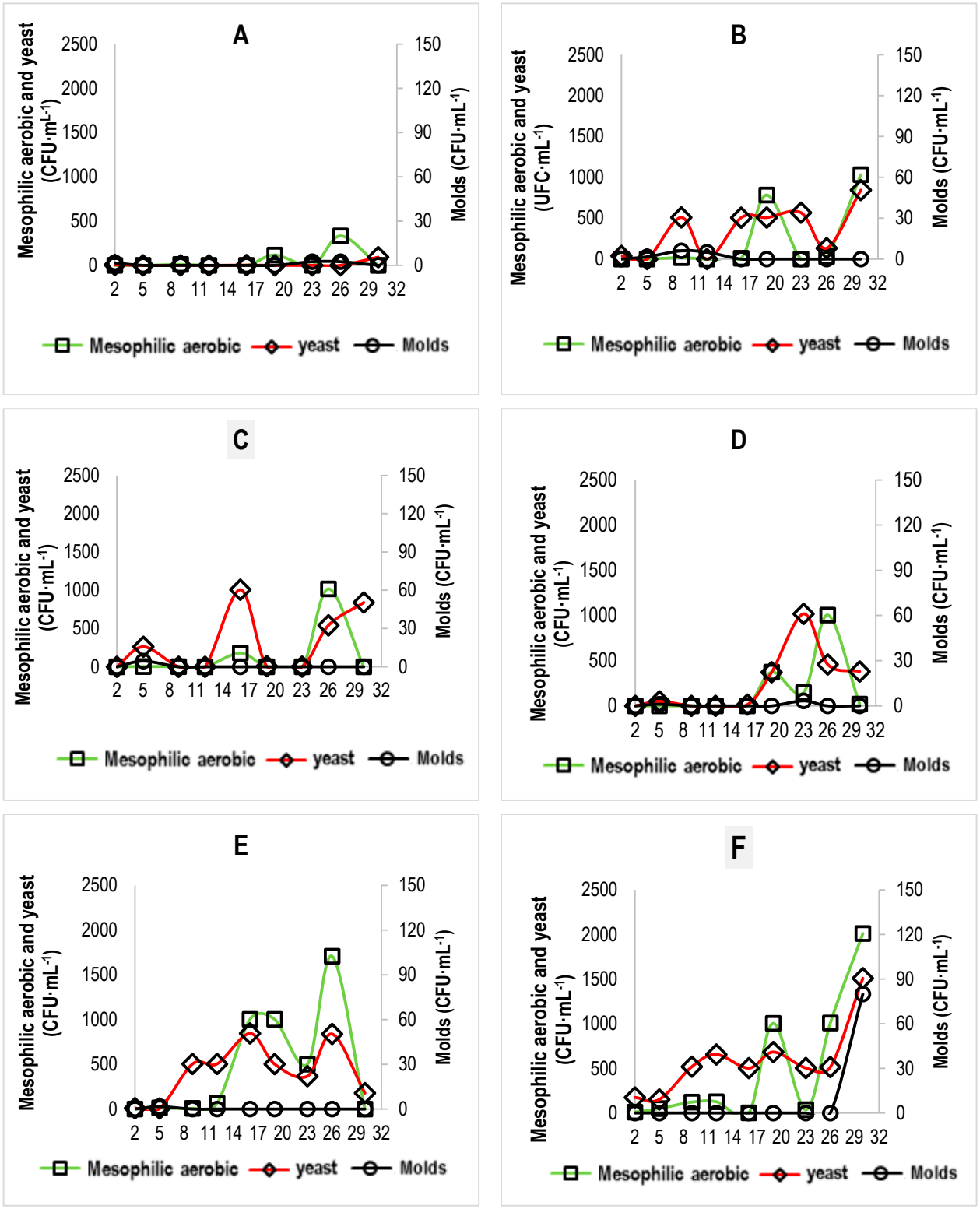

Figure 4. Evolution of the content of total mesophilic aerobic bacteria (□), yeasts (◊) and molds (○) in orange and carrot nectars treated by vacuum cooking. T1: 92 °C/3.3 min (**A**); T2: 90 °C/10.3 min (**B**); T3: 88 °C/32.7 min (**C**); T4: 70 °C/2.3 min (**D**); T5: 65 °C/11.4 min (**E**); T6: 60 °C/56.6 min (**F**). NEN standard limit < 10 CFU·mL^{-1} for total mesophilic aerobic microorganisms and <10 PUF·mL^{-1} for molds in pasteurized products: juices, pulps, concentrates, nectars, fruit and vegetable drinks.

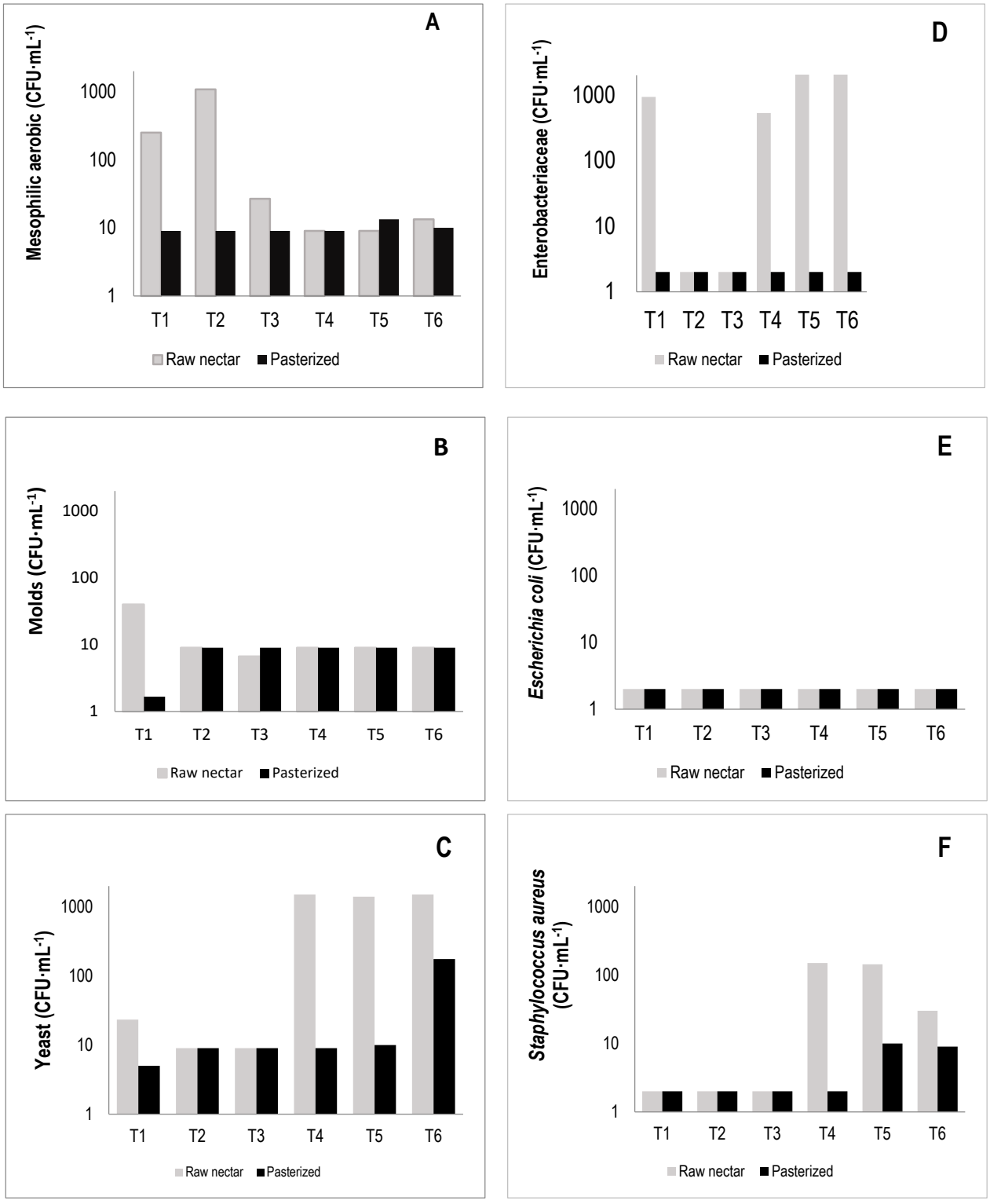

Figure 5. Effectiveness of the pasteurization process on the content of index microorganisms (**A**–**C**) and pathogenic microorganisms (**D**–**F**) of 6 treatments of orange and carrot nectar, treated by vacuum cooking. Evolution of the content of total mesophilic aerobic microorganisms (**A**), molds (**B**), yeasts (**C**), Enterobacteriaceae (**D**), *Escherichia coli* (**E**) and *Staphylococcus aureus* (**F**). T1: 92 °C/3.3 min, T2: 90 °C/10.3 min, T3: 88 °C/32.7 min, T4: 70 °C/2.3 min, T5: 65 °C/11.4 min, T6: 60 °C/56.6 min. INEN standard limit < 10 CFU·mL^{-1} for index microorganisms in pasteurized products and < 3 CFU·mL^{-1} for pathogenic microorganisms.

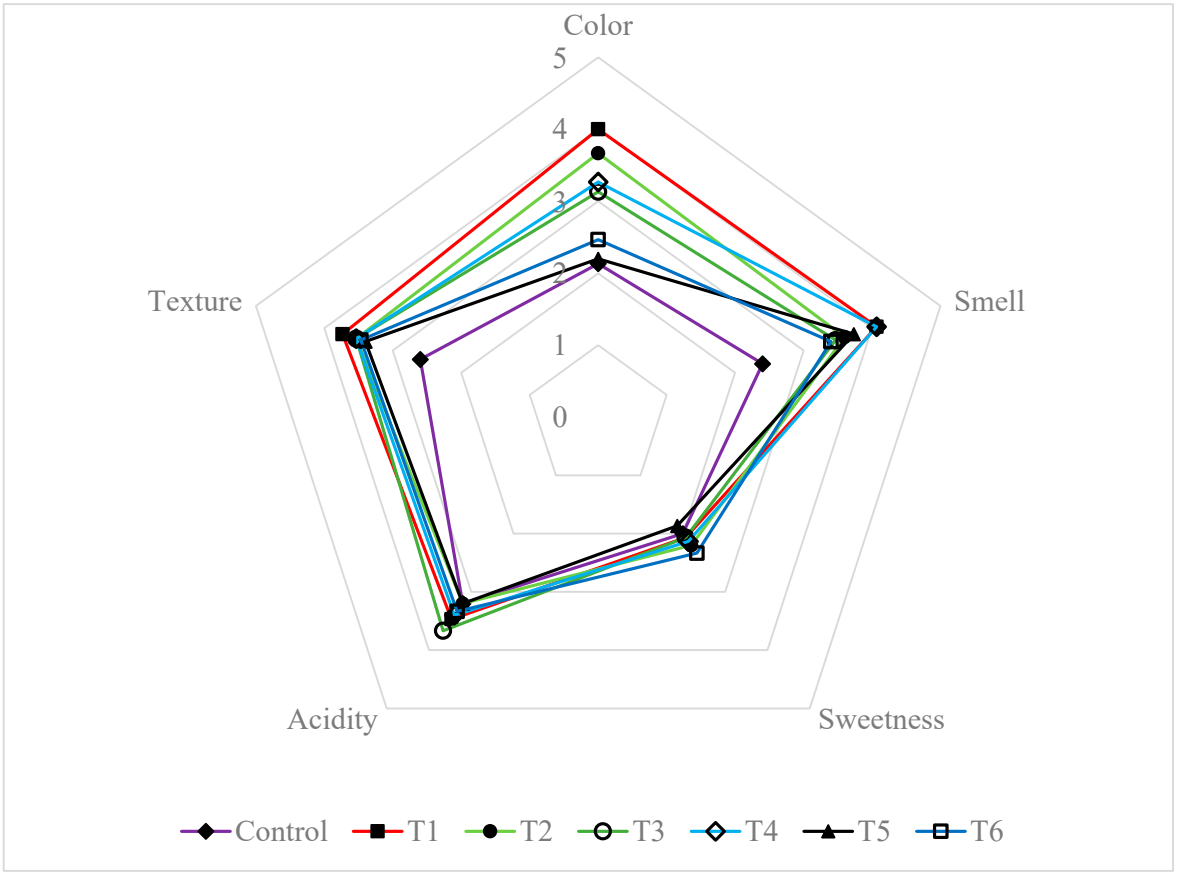

Figure 6. Descriptive parameters of sensory evaluation of orange and carrot nectars, treated by vacuum cooking. T1: 92 °C/3.3 min; T2: 90 °C/10.3 min; T3: 88 °C/32.7 min; T4: 70 °C/2.3 min; T5: 65 °C/11.4 min; T6: 60 °C/56.6 min.

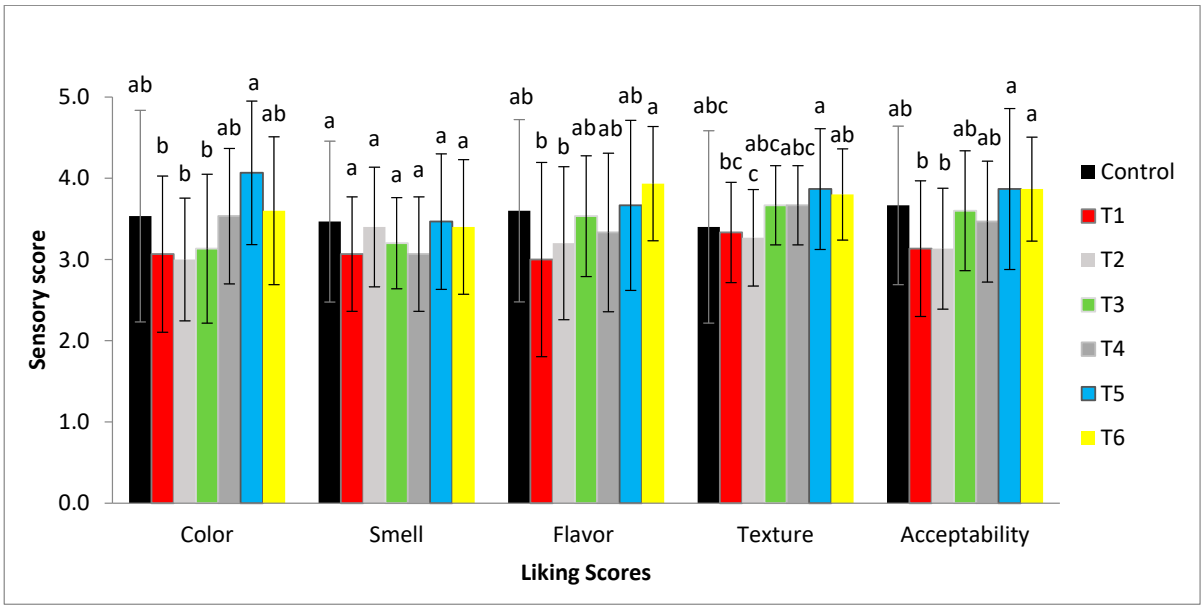

Figure 7. Hedonic parameters for sensory evaluation of orange and carrot nectars, treated by vacuum cooking. T1: 92 °C/3.3 min; T2: 90 °C/10.3 min; T3: 88 °C/32.7 min; T4: 70 °C/2.3 min; T5: 65 °C/11.4 min; T6: 60 °C/56.6 min, control. a, b, c: Significant differences ($p < 0.05$) between thermal treatments (T1, T2, T3, T4. T5, T6).

4. Conclusions

Aseptic juices were processed using different-temperature pasteurization, eradicating pathogenic bacteria in beverages. The pasteurization of orange and carrot nectars in vacuum conditions did not significantly modify their physicochemical properties (TSS, pH, acidity) and allowed for the better retention of nutrients such as vitamin C. In addition to preserving the color and smell of the nectar, it slowed down the microbial growth of aerobes, total mesophiles, molds and yeasts and reduced the microbial load of pathogens to permissible limits. The development of novel and emerging thermal treatment technologies has resulted from the food industry's effort to find solutions to produce healthy, safe, highly nutritious and long-shelf-life foods. As all processing technologies have advantages and disadvantages, adopting aseptic pasteurization in the food industry should be thoroughly considered to optimize all the involved parameters, such as temperature and time.

Author Contributions: Conceptualization, L.-S.W., J.B., I.A., R.S.A. and J.O.; methodology, L.-S.W., I.A., I.S., R.S.A. and J.B.; chemical analysis, L.-S.W., I.A., I.S., J.B. and R.S.A.; statistical analysis L.-S.W., I.A., I.S., J.M., C.V., R.S.A. and J.B.; field sampling and agronomical management, L.-S.W., J.O., A.Y. and I.A.; writing—original draft preparation, L.-S.W., I.A., I.S., M.J., J.B. and R.S.A.; writing—review and editing, L.-S.W., I.S., J.O., C.V. and R.S.A.; project administration, I.A., R.S.A., J.M. and J.O.; funding acquisition, I.A., R.S.A. and J.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Dirección de Investigación y Desarrollo from the Universidad Técnica de Ambato (Ecuador) for its financial support through its funding project 1371-CU-P-2014.

Data Availability Statement: Data are contained within the article.

Acknowledgments: Authors want to recognize Jessica Guevara and Jonathan Sánchez for their support in the experimental development of this research. The authors also wish to acknowledge Mario Álvarez and Mónica Silva for the use of the facilities and equipment belonging to the project "Canje de Deuda Ecuador-España" (AECID—Agencia Española de Cooperación Internacional para el Desarrollo).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Seifter, J.L. *Potassium Disorders Goldman's Cecil Medicine*, 24th ed.; Elsevier: Philadelphia, PA, USA, 2008; pp. 734–752.
2. Sadecka, J.; Polovka, M.; Kolek, E.; Belajova, E.; Tobolkova, B.; DAŠKO, L.; Durec, J.Á.N. Orange juice with pulp: Impact of pasteurization and storage on flavour, polyphenols, ascorbic acid and antioxidant activity. *J. Food Nutr. Res.* **2014**, *53*, 371–388.
3. Nadulski, R.; Grochowicz, J.; Sobczak, P.; Kobus, Z.; Panasiewicz, M.; Zawiślak, K.; Mazur, J.; Starek, A.; Żukiewicz-Sobczak, W. Application of Freezing and Thawing to Carrot (*Daucus carota* L.) Juice Extraction. *Food Bioprocess Technol.* **2014**, *8*, 218–227. [CrossRef]
4. Gabriel, A.A.; Albura, M.P.; Faustino, K.C. Thermal death times of acid-habituated Escherichia coli and Salmonella enterica in selected fruit bev-erages. *Food Control.* **2015**, *55*, 236–241. [CrossRef]
5. Wibowo, S.; Grauwet, T.; Santiago, J.S.; Tomic, J.; Vervoort, L.; Hendrickx, M.; Van Loey, A. Quality changes of pasteurised orange juice during storage: A kinetic study of specific parameters and their relation to colour instability. *Food Chem.* **2015**, *187*, 140–151. [CrossRef] [PubMed]
6. Kalia, P.K.M. Pectin Methylesterases: A Review. *J. Bioprocess. Biotech.* **2015**, *5*, 1000227. [CrossRef]
7. Tchango, J.T.; Tailliez, R.; Eb, P.; Njine, T.; Hornez, J. Heat resistance of the spoilage yeastsCandida pelliculosaandKloeckera apisand pasteurization values for some tropical fruit juices and nectars. *Food Microbiol.* **1997**, *14*, 93–99. [CrossRef]
8. Salomão, B.; Slongo, A.; Aragão, G. Heat resistance of Neosartorya fischeri in various juices. *LWT Food Sci. Technol.* **2007**, *40*, 676–680. [CrossRef]
9. Hommel, R.K. Gluconobacter. In *Encyclopedia of Food Microbiology*, 2nd ed.; Batt, C.A., Ed.; Academic Press: Cambridge, MA, USA, 2014; Volume 1, p. 994.
10. Juvonen, R.; Virkajärvi, V.; Priha, O.; Laitila, A. Microbiological spoilage and safety risks in non-beer beverages. *VTT Tied. Res. Notes* **2011**, *2599*, 107.
11. Nielsen, P.V.; Beuchat, L.R.; Frisvad, J.C. Growth of and fumitremorgin production by Neosartorya fischeri as affected by temperature, light, and water activity. *Appl. Environ. Microbiol.* **1988**, *54*, 1504–1510. [CrossRef]
12. Palaniappan, S.; Sastry, S.K.; Richter, E.R. Effects of Electroconductive Heat Treatment and Electrical Pretreatment on Thermal Death Kinetics of Selected Microorganisms. *Biotechnol. Bioeng.* **1992**, *39*, 225–232. [CrossRef]

13. Abraham, K.; Gürtler, R.; Berg, K.; Heinemeyer, G.; Lampen, A.; Appel, K.E. Toxicology and risk assessment of 5-Hydroxymethylfurfural in food. *Mol. Nutr. Food Res.* **2011**, *55*, 667–678. [CrossRef] [PubMed]
14. Rivas, A.; Rodrigo, D.; Martínez, A.; Barbosa-Cánovas, G.; Rodrigo, M. Effect of PEF and heat pasteurization on the physical–chemical characteristics of blended orange and carrot juice. *LWT Food Sci. Technol.* **2006**, *39*, 1163–1170. [CrossRef]
15. IDFA. Pasteurization. Consulted on 09/27/2016. 2016. Available online: https://www.idfa.org/news-views/media-kits/milk/pasteurization (accessed on 15 March 2024).
16. Burbano, J. Influence of Open and Vacuum Pasteurization on the Physicochemical Properties and Acceptability of Pineapple (*Ananas comosus* L.), Naranjilla (*Solanum quitoense* Lam.) and Borojo (*Borojoa patinoi* Cuatrec.) Nectar. Master's Thesis, Technical University of Ambato, Ambato, Ecuador, 2015.
17. *NTE INEN-ISO 2337*; Juices, Pulps, Concentrates, Nectars, Fruit and Vegetable Drinks. INEN, Ecuadorian Technical Standard: Quito, Ecuador, 2008; Requirements. p. 15.
18. Rajashekhara, E.; Suresh, E.; Ethiraj, S. Note: Influence of different heating media on thermal resistance of Neosartorya fischeri isolated from papaya fruit. *J. Appl. Bacteriol.* **1996**, *81*, 337–340. [CrossRef]
19. *NTE INEN-ISO 1842*; Vegetable and Fruit Products. Determination of pH (IDT). INEN, Ecuadorian Technical Standard: Quito, Ecuador, 2013; p. 4.
20. Aleman, R.S.; Cedillos, R.; Page, R.; Olson, D.; Aryana, K. Physico-chemical, microbiological, and sensory characteristics of yogurt as affected by ingredients that help treat leaky gut. *J. Dairy Sci.* **2023**, *106*, 3868–3883. [CrossRef] [PubMed]
21. *NTE INEN-ISO 2173*; Vegetable and Fruit Products. Determination of Soluble Solids. Refractometric Method (IDT). INEN, Ecuadorian Technical Standard: Quito, Ecuador, 2013; p. 9.
22. Fuentes, J.A.M.; Gil, M.D.J.; Fernández, H.Z.; Montero-Fernández, I.; Martín-Vertedor, D.; Yadav, A.; Aleman, R.S. Impact of 5′ Adenosine Monophosphate, Potassium Chloride, and Glycine on the Physicochemical and Sensory Characteristics of Sodium-Reduced Chicken. *Dietetics* **2024**, *3*, 87–97. [CrossRef]
23. Miceli, A.; Vetrano, F.; Sabatino, L.; D'Anna, F.; Moncada, A. Influence of Preharvest Gibberellic Acid Treatments on Postharvest Quality of Minimally Processed Leaf Lettuce and Rocket. *Horticulturae* **2019**, *5*, 63. [CrossRef]
24. He, O.; Zhang, Y.; Wang, P.; Liu, L.; Wang, Q.; Yang, N.; Li, W.; Champagne, P.; Yu, H. Experimental and Kinetic Study on the Production of Furfural and HMF from Glucose. *Catalysts* **2021**, *11*, 11. [CrossRef]
25. *NTE INEN 1529-5*; Microbiological Control of Food. Determination of the Amount of Mesophilic Aerobic Microorganisms. INEN, Ecuadorian Technical Standard: Quito, Ecuador, 2006; REP. p. 6.
26. *NTE INEN 1529-10*; Microbiological Control of Food. Molds and Viable Yeasts. Plate Count by Deep Seeding. INEN, Ecuadorian Technical Standard: Quito, Ecuador, 2013; p. 6.
27. 3M Petrifilm Interpretation Guides. 2016. Available online: https://solutions.3m.co.uk/wps/portal/3M/en_GB/FoodSafetyEU/FoodSafety/EducationTraining/BrochuresDownloads/PetrifilmInterpretationGuides/ (accessed on 7 March 2024).
28. AOAC. *AOAC Official Method 2003.07 for Counting Staphylococcus aureus in Prepared Processed Foods*; AOAC International: Rockville, MD, USA, 2003.
29. AOAC. *AOAC RI 0401101 Official Method for Counting Listeria Monocytogenes*; AOAC International: Rockville, MD, USA, 2002.
30. AOAC. *AOAC Official Method 991.14 for Counting E. coli/Coliforms*; AOAC International: Rockville, MD, USA, 2002.
31. Aguilar-Rosas, S.; Ballinas-Casarrubias, M.; Nevarez-Moorillon, G.; Martin-Belloso, O.; Ortega-Rivas, E. Thermal and pulsed electric fields pasteurization of apple juice: Effects on physicochemical properties and flavour compounds. *J. Food Eng.* **2007**, *83*, 41–46. [CrossRef]
32. Zhao, L.; Wang, S.; Liu, F.; Dong, P.; Huang, W.; Xiong, L.; Liao, X. Comparing the effects of high hydrostatic pressure and thermal pasteurization combined with nisin on the quality of cucumber juice drinks. *Innov. Food Sci. Emerg. Technol.* **2013**, *17*, 27–36. [CrossRef]
33. Igual, M.; García-Martínez, E.; Camacho, M.; Martínez-Navarrete, N. Effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice. *Food Chem.* **2010**, *118*, 291–299. [CrossRef]
34. Finlayson, G.D.; Schaefer, G. Hue that is invariant to brightness and gamma. In Proceedings of the 12th British Machine Vision Conference, Manchester, UK, 10–13 September 2001.
35. Jin, Z.; Min, S.; Yeom, H.; Zhang, Q. Commercial-Scale Pulsed Electric Field Processing of Orange Juice. *J. Food Sci.* **2003**, *68*, 1265–1271. [CrossRef]
36. Choi, M.; Kim, G.; Lee, H. Effects of ascorbic acid retention on juice color and pigment stability in blood orange (*Citrus sinensis*) juice during refrigerated storage. *Food Res. Int.* **2002**, *35*, 753–759. [CrossRef]
37. Lee, H.S.; Coates, G.A. Effect of thermal pasteurization on Valencia orange juice color and pigments. *LWT Food Sci. Technol.* **2003**, *36*, 153–156. [CrossRef]
38. Silva, F.M.; Silva, C.L.M. Colour changes in thermally processed cupuaçu (*Theobroma grandiflorum*) puree: Critical times and kinetics modelling. *Int. J. Food Sci. Technol.* **1999**, *34*, 87–94. [CrossRef]
39. Cortés, C.; Esteve, M.J.; Frígola, A. Color of orange juice treated by High Intensity Pulsed Electric Fields during refrigerated storage and comparison with pasteurized juice. *Food Control.* **2008**, *19*, 151–158. [CrossRef]

40. Burdurlu, H.S.; Koca, N.; Karadeniz, F. Degradation of vitamin C in citrus juice concentrates during storage. *J. Food Eng.* **2006**, *74*, 211–216. [CrossRef]
41. Aarnisalo, K.; Tallavaara, K.; Wirtanen, G.; Maijala, R.; Raaska, L. The hygienic working practices of maintenance personnel and equipment hygiene in the Finnish food industry. *Food Control.* **2006**, *17*, 1001–1011. [CrossRef]