

Article

Effect of Sous Vide Heat Treatment and Packaging Methods on the Microbiological Quality of Red Peppers (*Capsicum annuum* L.)

Paweł Hanus ¹, Grażyna Jaworska ¹, Miroslava Kačániová ^{2,3}, Natalia Szarek ⁴ and Karolina Pycia ^{1,*}

¹ Department of Food Technology and Human Nutrition, University of Rzeszów, St. Zelwerowicza 4, 35-601 Rzeszów, Poland; phanus@ur.edu.pl (P.H.) gjaworska@ur.edu.pl (G.J.)

² Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture, Trieda Andreja Hlibnku 2, 949 76 Nitra, Slovakia; miroslava.kacaniova@gmail.com

³ School of Medical & Health Sciences, University of Economics and Human Sciences in Warsaw, Okopowa 59, 01-043 Warszawa, Poland; m.kacaniova@vizja.pl

⁴ Doctoral School of the University of Rzeszów, University of Rzeszów, Rejtana 16C Street, 35-959 Rzeszów, Poland; nataliasz@dokt.ur.edu.pl

* Correspondence: kpycia@ur.edu.pl

Abstract: This study aimed to evaluate the effect of sous vide heat treatment and different packaging methods on the microbiological quality of red peppers (*Capsicum annuum* L.) during their refrigerated storage. Peppers of the *Roberta* variety were used in the study. The heat treatment of the peppers was carried out under vacuum conditions for all the packaging methods. ATM (unaltered gas atmosphere) and MAP (modified atmosphere packaging) products were repackaged under aseptic conditions into a suitable gaseous atmosphere after heat treatment and cooling into an unaltered atmosphere (ATM), and into a modified atmosphere (MAP: 30% CO₂, 70% N₂). The sous vide thermal treatment was performed in three variations: 30 min/60 °C, 15 min/70 °C, and 8 min/80 °C. The peppers were stored at 3 ± 1 °C until the bacterial count first exceeded 4 log CFU/g, but no longer than 24 days. The pH, TVC, Enterobacteriaceae count, and microscopic filamentous fungi count were determined in the products. A qualitative analysis of microbial isolates was also carried out using MALDI-TOF mass spectrometry. Sous vide heat treatment reduced the number of TVC (total viable count) (<2 to 2.35 log CFU/g), Enterobacteriaceae (<2 log CFU/g), and the number of microscopic filamentous fungi (<2 log CFU/g) compared with control (TVC 3.54–3.86 log CFU/g). The use of heat treatment combined with the packaging of culinary products effectively extends the shelf life of sous vide peppers, from 3 to 6 days for raw peppers to 15–24 days for sous vide peppers depending on the heat treatment and packaging method. All the packaging methods allowed the product to remain microbiologically safe (TVC below 4 log CFU/g) for 24 days for 30 min/60 °C and 15 min/70 °C treatments, and VAC packaging for 8 min/80 °C treatments. Bacterial identification by MALDI-TOF MS revealed that the predominant families were Pseudomonadaceae, Yersiniaceae, and Staphylococcaceae. Depending on the method of sous vide thermal treatment, it showed differences in the percentage of bacterial families in the culinary products. The proportions in the proportion of Gram+ and Gram- bacteria also changed. The use of sous vide processing in combination with appropriate packaging methods can significantly extend the shelf life of products obtained from peppers.

Keywords: microbial safety; packaging method; pepper bell; MALDI-TOF; sous vide



Academic Editors: Alejandro Rodríguez Pascual, Alessandra Biancolillo, Marco Iammarino and Oana Bianca Oprea

Received: 16 October 2024

Revised: 15 January 2025

Accepted: 21 January 2025

Published: 27 January 2025

Citation: Hanus, P.; Jaworska, G.; Kačániová, M.; Szarek, N.; Pycia, K. Effect of Sous Vide Heat Treatment and Packaging Methods on the Microbiological Quality of Red Peppers (*Capsicum annuum* L.). *Appl. Sci.* **2025**, *15*, 1303. <https://doi.org/10.3390/app15031303>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The growing demand for convenience foods has led to the development of an assortment of different ready-to-eat and semi-processed foods. Vegetables, including red peppers (*Capsicum annuum* L.), are essential components of a balanced diet, providing carbohydrates, including dietary fibre, vitamins, and minerals [1]. However, their long-term storage poses the risk of nutritional loss and the appearance of microbial contamination. Therefore, the optimisation of thermal processing and packaging methods is crucial to ensure food safety and adequate quality [2–5]. Red pepper (*Capsicum annuum* L.) is a widely consumed vegetable with a wide range of culinary and industrial uses belonging to the Solanaceae family characterised by intense colour, distinctive flavour, and high nutritional value, including high levels of carotenoids, polyphenols, antioxidants, and capsaicinoids. Particularly noteworthy is the high level of vitamin C, up to 277 mg/100 g fresh weight [6–8], which plays a crucial role in various metabolic processes and acts as a powerful antioxidant. The carotenoids contained in red peppers, such as β -carotene and capsanthin, contribute to its vibrant colour and health benefits, including anti-inflammatory and anticancer properties [9–11].

Ensuring the microbiological quality of food is crucial for consumer safety and product shelf life. Traditional preservation methods often include heat treatment and various packaging techniques to inhibit microbial growth. In recent years, sous vide heat treatment has gained attention as an innovative food processing method for improved quality and safety. The sous vide method, combined with appropriate packaging, can effectively reduce microbial contamination and extend the shelf life of vegetables. Vacuum packaging (VAC) and modified atmosphere packaging (MAP) are commonly used to increase the microbiological stability of sous vide products. These packaging methods reduce exposure to oxygen, thereby inhibiting the growth of aerobic spoilage organisms and pathogens [12]. Thermal processing methods, including sous vide, significantly affect the nutritional quality of vegetables. Sous vide, a low-temperature, long-time cooking (LTLT) method, involves vacuum-sealing food and cooking it in a water bath at strictly defined temperatures [13]. In the context of food preservation, sous vide goes beyond traditional cooking to encompass processes aimed at increasing shelf life and safety. According to Baldwin [14], sous vide processing allows processing at lower temperatures for longer periods, effectively inactivating pathogens while preserving food products' sensory and nutritional qualities. There is little or no scientific evidence to support any predictive model for the inactivation of foodborne pathogens in vegetables at cooking temperatures at or below 55 °C, so the safety of products cooked by sous vide at temperatures below 55 °C cannot be guaranteed [13]. In contrast, according to a study by Stringer and Metris [15], vegetables should be cooked at 70 °C for at least two minutes to counter the risk of foodborne pathogens such as *L. monocytogenes*. A study by Singh et al. [16] demonstrated the effectiveness of sous vide in reducing the microbial load in various vegetables, highlighting its potential application to red peppers. Studies by Iborra-Bernard et al. [17] and Stankov et al. [18] indicate that sous vide processing can significantly improve the microbiological quality of red peppers. Controlled temperature and vacuum conditions reduce the risk of contamination by pathogens such as *Listeria monocytogenes* and *Escherichia coli*. In addition, sous vide processing helps preserve red peppers' structural integrity and nutrients, making it a suitable method to produce ready-to-eat high-quality, safe, and nutritious vegetable products.

The use of sous vide heat treatment combined with packaging methods such as VAC and MAP offers opportunities to improve the microbiological quality and shelf life of red peppers. With sous vide processing, targeted microbial inactivation can be achieved without excessive heat damage, while appropriate packaging can inhibit post-processing contamination and slow down microbial growth during storage. A study by Onyeaka

et al. [19] showed that vacuum-packed sous vide-treated vegetables exhibited significantly lower microbial counts compared to those packaged in air. Similarly, MAP has been shown to extend the shelf life of sous vide products by creating an environment that is unfavourable to spoilage organisms [13]. Furthermore, research by Souza et al. [20] and Chem et al. [21] sheds new light on the safety and applicability of different packaging materials than before. The use of biodegradable food packaging with bacterial growth inhibitors may bring additional benefits to food producers and consumers. Thus, further research is needed to optimise processing parameters and explore the potential of sous vide in combination with other preservation methods to develop innovative and safe food products.

The aim of this study was to analyse the effect of sous vide heat treatment parameters and different packaging methods on selected physicochemical parameters and the microbiological quality of red peppers during cold storage. The obtained research results will allow for the assessment of whether the combined methods of red pepper preservation (sous vide method conditions and packaging method) will allow for maintaining the appropriate microbiological quality during cold packaging and will indicate possible factors for modification. Sous vide heat treatment combined with appropriate packaging methods (ATM, VAC, and MAP) can effectively reduce the number of microorganisms such as TVC and Enterobacteriaceae, as well as the number of microscopic filamentous fungi, compared to raw peppers, and can also bring economic and environmental benefits such as reduced food losses and lower storage costs.

2. Materials and the Experimental Design

2.1. Study Material

The research material was raw red annual peppers (*Capsicum annuum* L.) of the *Roberta* variety and the culinary products obtained from them. The fruiting bodies of red peppers (Subcarpathian Voivodeship, Poland) were intended for the study. The fruiting bodies intended for the study were fresh, with the shape and uniform colour characteristic of the cultivar, with a size in the 200–300 g range. Approximately 20 kg of peppers was used for the study. After pre-sorting (e.g., eliminating fruiting bodies that were too small or too large and not correctly coloured; the maturity was assessed visually), the peppers were divided into three equal parts for the three packaging methods. Each packaging method included four treatment options: no heat treatment (control) and three sous vide heat treatment options. Immediately after purchase, the fresh vegetables were pre-treated, which included the removal of the seed nests, washing in running cold water and drying, and slicing into oblong rectangular pieces of 20 ± 4 g (dry weight range); their length and width may have varied but was approximately $2.5 \text{ cm} \times 4 \text{ cm}$.

The heat treatment of the peppers was carried out under vacuum conditions for all the packaging methods (ATM, VAC, and MAP). The VAC products were cooled after heat treatment and destined for storage. The ATM and MAP products were repackaged under aseptic conditions into a suitable gaseous atmosphere after processing and cooling (ATM and MAP). The packaging material for packaging under an unchanged gas atmosphere was string pouches made of 100 μm thick polyethylene (PE) transparent film (Inter Arma, Rudawa, Poland). A 70 μm thick, 100×150 mm double-layer polyamide/polyethylene (PA/PE) (Inter Arma, Rudawa, Poland) transparent film was used for the vacuum packaging of culinary products. An RM Gastro VBN-18SB (RM Gastro, Prague, Czech Republic) packaging machine was used for the VAC packaging of culinary products. The following parameters were used during packaging: vacuum generation time 5 s, vacuum 0.9 MPa, and sealing time 13 s. The VAC packaging conditions were experimentally determined using an optimum pressure and sealing time that did not damage the culinary products

during the packaging process. For the modified gas atmosphere packaging of culinary products, a two-layer polyamide/polyethylene (PA/PE) film with a thickness of 70 μm and dimensions of 100 mm $\times$ 150 mm was used. An RM Gastro VBN-18SB (RM Gastro, Prague, Czech Republic) packaging machine was used for the MAP packaging of culinary products. BIOGON C30 food gas (Linde, Dublin, Ireland) had a composition of 30% CO_2 and 70% N_2 . The following parameters were used during packaging: pressure build-up 1 MPa, gas injection 10 s, and sealing time 10 s. The MAP packing conditions were experimentally determined so that the gas filled the pack's volume.

Sous vide hydrothermal treatment was carried out using a water circulator (CASO 2000, Arnsberg, Germany). Three variants of hydrothermal treatment of peppers were used for each packaging method: 30 min at 60 $^{\circ}\text{C}$ packed in an unaltered gas atmosphere (30 min/60 $^{\circ}\text{C}$ ATM), vacuum packed (30 min/60 $^{\circ}\text{C}$ VAC), and packed in a modified gas atmosphere (30 min/60 $^{\circ}\text{C}$ MAP); 15 min at 70 $^{\circ}\text{C}$ in an unchanged gas atmosphere (15 min/70 $^{\circ}\text{C}$ ATM), vacuum packed (15 min/70 $^{\circ}\text{C}$ VAC), and packaged in a modified gas atmosphere (15 min/70 $^{\circ}\text{C}$ MAP); and 8 min at 80 $^{\circ}\text{C}$ in an unchanged gas atmosphere (8 min/80 $^{\circ}\text{C}$ ATM), vacuum packed (8 min/80 $^{\circ}\text{C}$ VAC), and packaged in a modified gas atmosphere (8 min/80 $^{\circ}\text{C}$ MAP). The aforementioned hydrothermal treatments and packaging methods, together with the raw peppers' packaging, resulted in 12 test samples. Each test sample was prepared in 54 replicates (9 test dates were planned, each with $n = 6$). The dry weight of the raw fruiting bodies on the first day of storage ranged from 8.14 to 8.46%, while after the sous vide treatment, it ranged from 7.82% to 9.31%. The packaging method and storage time did not have a statistically significant effect ($p \geq 0.05$) on the dry matter content of the raw products. After the sous vide treatment, the peppers were immediately cooled to 4 $^{\circ}\text{C}$ in ice water. The packaged samples of raw peppers and sous vide-treated peppers were stored under refrigeration at a temperature of not more than 4 $^{\circ}\text{C}$ (3 ± 1 $^{\circ}\text{C}$) (FKv 36110, Liebherr, Donau, Germany) until analysis. The raw and sous vide peppers were assessed on day 1 of storage and every 3 days thereafter until day 24 of the experiment.

2.2. Study Methods

2.2.1. Determination of Active Acidity

The active acidity (pH) of the products was determined using a pH-METR CP-411 (Elmetron, Zabrze, Poland) [22]. The test was carried out in 3 replicates.

2.2.2. Microbiological Quality Assessment

The microbiological quality of the raw and cooked pepper fruiting bodies was assessed every 3 days until the bacterial counts first exceeded 4 log CFU/g, but not longer than 24 days. The study assumed that bacterial counts > 4 log CFU/g resulted in unsatisfactory product quality. The limit was established in accordance with the UK Health Security Agency and the NSW Food Authority. The microbiological analysis included the determination of the total viable count (TVC), the number of Enterobacteriaceae, and the number of microscopic filamentous fungi. In addition, bacterial identification was performed from the bacterial cells obtained by a MALDI-TOF MS Biotyper mass spectrometer analysis by measuring unique bacterial proteins.

2.2.3. Preparation of Samples for Microbiological Tests

The material for microbiological tests was secured in 100 mL containers. Samples weighing 5.00 ± 0.1 g were cut from the pepper pieces and transferred to sterile disposable containers. A total of 45 mL of saline (0.9% NaCl) was added to the sample containers and mixed in a BagMixer[®] 400S homogeniser (stomacher) (Interscience, Saint Nom la Br  t  che, France). Serial dilutions from 10^{-1} to 10^{-5} were prepared from the solutions. To determine the total viable count (TVC), the samples were cultured in Trypticasein Soy

Lab-Agar medium (TSA, Biocorp, Paris, France) according to [23]. The samples were incubated for 48 h at 37 °C under aerobic conditions. For the isolation of Enterobacteriaceae, Violet Red Bile Glucose Agar (VRBL, Biocorp, Paris, France) was used according to ISO 11133:2014 [24]. The inoculated plates were incubated at 37 °C for 24 h. To determine the number of microscopic filamentous fungi, Malt Extract Agar (MEA, Sigma-Aldrich, St. Louis, MO, USA) was used according to [25]. The plates were incubated at 25 °C for 48 h. Microbiological tests were performed in triplicate for each sample and each medium. After incubation, the bacterial colonies of the plates were counted, with plate counts ranging from 15 to 150 colonies for Enterobacteriaceae and 30 to 300 colonies for TVC [26]. Plates for strain identification were stored in a cold room (FKv 36110, from Liebherr, Donau, Germany) at 3 °C $\pm$ 1 °C for up to 24 h.

2.2.4. Mass Spectrometry Identification of Isolates

A qualitative analysis of microbial isolates was performed using the method described by Kačániová et al. [27] using MALDI-TOF mass spectrometry (Bruker Daltonics, Bremen, Germany). The bacterial biomass of the microbial medium was transferred to 300 µL of distilled water. Then, 900 µL of ethanol was added and mixed using Vortex (Vortex Classic, Velp Scientifica, Usmate, Italy). The bacterial suspension in the ethanol solution was centrifuged (MPW-150R, MPW MED. INSTRUMENTS, Warsaw, Poland) for 2 min at 14,000 rpm (RCF = 22,570 $\times$ g) at room temperature (19–21 °C). The supernatant was discarded and the bacterial biomass was allowed to dry. In total, 30 µL of 70% formic acid and 30 µL of acetonitrile were added and mixed using a vortex. The tubes were centrifuged for 2 min at 14,000 rpm (RCF = 22,570 $\times$ g) at room temperature (19–21 °C). A total of 1 µL of supernatant was applied to a 96-well metal plate, each sample to a separate well. After drying at room temperature (19–21 °C), each well was coated with 1 µL of alpha-cyano-4-hydroxycinnamic acid (HCCA) and allowed to dry at room temperature. The metal plate with the overlaid samples was placed in the measuring chamber of the apparatus, the air was then removed to create a vacuum and the plate was exposed to a laser beam. Under their action, bacterial proteins were desorbed and ionised, and the ionised peptides were accelerated in a strong electric field by measuring their transit time. Based on the resulting distribution of peptides and their molecular weights, charge, and time of flight, the MALDI-TOF system generated spectrophotometric spectra. The peak spectra corresponded to ions with different mass–charge ratios. The spectrum measurements were automatically compared with a database of microbial reference spectra. The generated spectra were analysed on a MALDI-TOF Microflex LT instrument (Bruker Daltonics, Bremen, Germany) using the Flex Control 3.4 software.

2.2.5. Statistical Analysis

The results of the study were statistically processed using the Statistica v.13.1 software (Stat-Soft). The results of the study are presented as means and standard deviations. The effects of storage time and packaging method on the pH parameter were evaluated by one-way and multivariate analysis of variance (ANOVA and MANOVA) (using Fisher's NIR post hoc tests). The effect of storage time on the microbiological parameters of the peppers was evaluated by one-way analysis of variance (ANOVA) (using Fisher's NIR post hoc tests). The differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. The pH Value of the Culinary Products Tested

The pH values of the raw fruiting bodies after culinary processing and during the refrigerated storage of the culinary products are shown in Table 1.

Table 1. pH values in raw peppers and after sous vide heat treatment during storage.

Type of Packaging	Storage Day	Control	Sous Vide			<i>p</i> -Value
			30 min/60 °C	15 min/70 °C	8 min/80 °C	
ATM	1 day	4.94 ^{a,b,A} ± 0.05	4.83 ^{d,e,f,g,h,A,B} ± 0.02	4.76 ^{g,h,B} ± 0.14	4.93 ^{g,A} ± 0.00	0.052
VAC		5.09 ^{b,A} ± 0.1	4.91 ^{h,C} ± 0.03	4.73 ^{f,g,h,B} ± 0.09	4.90 ^{e,f,g,A} ± 0.03	0.002
MAP		4.99 ^{a,b,A} ± 0.02	4.81 ^{c,d,e,f,g,B} ± 0.1	4.87 ^{i,B} ± 0.02	4.79 ^{b,c,B} ± 0.04	0.010
ATM	3 days	4.92 ^{a,b,A} ± 0.03	4.82 ^{d,e,f,g,A} ± 0.02	4.72 ^{f,g,h,B} ± 0.09	4.90 ^{e,f,g,A} ± 0.04	0.006
VAC		4.92 ^{a,b,A} ± 0.1	4.89 ^{g,h,A,B} ± 0.06	4.78 ^{h,i,B} ± 0.04	4.88 ^{d,e,f,g,A,B} ± 0.07	0.172
MAP		5.04 ^{a,b,A} ± 0.08	4.86 ^{f,g,h,B} ±0.07	4.78 ^{f,i,B,C} ± 0.02	4.74 ^{a,b,C} ± 0.06	0.001
ATM	6 days	nt	4.77 ^{a,b,c,d,e,B} ± 0.04	4.70 ^{d,e,f,g,h,B} ± 0.12	4.91 ^{f,g,A} ± 0.04	0.011
VAC		nt	4.85 ^{e,f,g,h,A,B} ± 0.07	4.70 ^{e,f,g,h,B} ± 0.09	4.86 ^{d,e,f,A} ± 0.06	0.073
MAP		4.87 ^{a,A} ± 0.24	4.75 ^{a,b,c,d,A} ± 0.04	4.71 ^{e,f,g,h,A} ± 0.06	4.70 ^{a,A} ± 0.02	0.425
ATM	9 days	nt	4.76 ^{a,b,c,d,B} ± 0.06	4.67 ^{a,b,c,d,e,f,g,C} ± 0.00	4.89 ^{d,e,f,g,A} ± 0.04	0.011
VAC		nt	4.78 ^{a,b,c,d,e,f,A,B} ± 0.04	4.72 ^{f,g,h,B} ± 0.07	4.85 ^{c,d,e,f,A} ± 0.04	0.057
MAP		nt	4.77 ^{a,b,c,d,e,A} ± 0.08	4.67 ^{a,b,c,d,e,f,g,A} ± 0.02	4.72 ^{a,b,A} ± 0.00	0.356
ATM	12 days	nt	4.75 ^{a,b,c,d,B} ± 0.05	4.65 ^{a,b,c,d,e,f,C} ± 0.03	4.84 ^{c,d,e,A} ± 0.04	0.004
VAC		nt	4.79 ^{a,b,c,d,e,f,A,B} ± 0.04	4.71 ^{e,f,g,h,B} ± 0.07	4.87 ^{d,e,f,g,A} ± 0.01	0.016
MAP		nt	4.75 ^{a,b,c,d,A} ± 0.06	4.59 ^{a,b,B} ± 0.08	4.71 ^{a,A} ± 0.01	0.026
ATM	15 days	nt	4.71 ^{a,A} ± 0.04	4.64 ^{a,b,c,d,e,f,A} ± 0.06	4.69 ^{a,A} ± 0.11	0.529
VAC		nt	4.73 ^{a,b,c,B} ± 0.06	4.69 ^{c,d,e,f,g,h,B} ± 0.03	4.83 ^{c,d,A} ± 0.04	0.018
MAP		nt	4.79 ^{a,b,c,d,e,f,A} ± 0.07	4.65 ^{a,b,c,d,e,f,B} ± 0.03	4.69 ^{a,B} ± 0.04	0.032
ATM	18 days	nt	4.78 ^{a,b,c,d,e,f,A} ± 0.06	4.62 ^{a,b,c,d,e,B} ± 0.05	nt	0.024
VAC		nt	4.72 ^{a,b,A,B} ± 0.05	4.67 ^{a,b,c,d,e,f,g,B} ± 0.02	4.74 ^{a,b,A} ± 0.01	0.078
MAP		nt	4.79 ^{a,b,c,d,e,f,A} ± 0.06	4.67 ^{a,b,c,d,e,f,g,B} ± 0.02	4.70 ^{a,B} ± 0.04	0.025
ATM	21 days	nt	4.75 ^{a,b,c,d,A} ± 0.07	4.59 ^{a,b,c,B} ± 0.04	nt	0.026
VAC		nt	4.72 ^{a,b,A} ± 0.02	4.68 ^{b,c,d,e,f,g,A} ± 0.03	4.72 ^{a,b,A} ± 0.02	0.182
MAP		nt	4.76 ^{a,b,c,d,A} ± 0.02	4.62 ^{a,b,c,d,e,B} ± 0.01	nt	<0.001
ATM	24 days	nt	4.83 ^{d,e,f,g,h,B} ± 0.02	4.58 ^{a,A} ± 0.03	nt	<0.001
VAC		nt	4.73 ^{a,b,c,A} ± 0.03	4.69 ^{c,d,e,f,g,h,A} ± 0.03	4.73 ^{a,b,A} ± 0.02	0.164
MAP		nt	4.80 ^{b,c,d,e,f,A} ± 0.05	4.60 ^{a,b,c,d,B} ± 0.05	nt	0.010
		Control	Sous vide			
			30 min/60 °C	15 min/70 °C	8 min/80 °C	
Type of packaging		0.129	<0.001	<0.001	<0.001	
Storage day		-	0.630	<0.001	-	
Type of packaging × Storage day		0.266	0.062	0.230	0.002	

Explanations: nt—samples were not tested due to exceeding the established limit set of 4 log CFU/g; ^{a,b,c,...}—values in the columns marked with different lowercase letters are significantly different $p < 0.05$; ^{A,B,C,...}—values in the rows marked with different capital letters are significantly different $p < 0.05$.

The pH value on the first day of storage of the raw pepper and after cooking ranged from 4.73 to 5.09. The heat treatment of the peppers and ATM packaging did not cause significant changes in the pH value, in contrast to the heat treatment and VAC and MAP packaging, which contributed to a significant reduction in the pH value on the first day of storage, by 2–7%. During the storage of the culinary products, a systematic reduction in pH values was found regardless of the heat treatment and packaging method. In the raw products at the end of storage, depending on the packaging method, the pH value varied between 4.87 and 4.92, in products after 30 min/60 °C treatment 4.73–4.83, after 15 min/70 °C treatment 4.58–4.69, and after 8 min/80 °C treatment 4.69–4.73. The packaging method significantly affected the pH value in all the thermal treatments, while it was not significant for the control sample. The day of storage significantly affected the pH parameter only in the case of the 15 min/70 °C

treatment, but no significant effect was found ($p = 0.102$). The effect of heat treatment on pH levels is also confirmed by a study by Dimassi et al. [28] on pastes made from sweet peppers. The authors found a decrease in pH values after heat treatment (6 h for 70 °C) compared to non-heat-treated paste from 5.52 to 5.41. In contrast, Rodini et al. [29] found no change in pH values during refrigerated storage (for a period of 12 days) of sweet pepper variety pastes subjected to mild heat treatment (45 °C for 3 min). Bozkurt and Erkmén [30], investigating the effect of preparation on the quality of pastes from hot pepper varieties, noted that pH decreased in the first 14 days and then increased in some cases, which could be related to the development of lactic fermentation bacteria, which initially lower pH by producing organic acids, but form alkaline compounds during prolonged storage. Another explanation was presented in the paper by Dos Reis et al. [31], where the authors reported that the pH of broccoli and cauliflower florets decreased by half a point after sous vide processing (90 °C, 20 min) compared to fresh samples. This was probably due to some cell wall ruptures that released internal acids.

3.2. Evaluation of the Total Viable Count in Raw Peppers and Culinary Products During Storage

Table 2 shows the results of the total viable count (TVC) for the raw peppers and culinary peppers packaged in an unaltered atmosphere (ATM), vacuum (VAC), and modified gas atmosphere (MAP). In the raw peppers, on the first day of the experiment, the TVC ranged from 3.54 to 3.86 log CFU/g and did not significantly depend on the packaging method ($p > 0.05$). During the storage of the culinary products, except 15 min/70 °C, the method of packaging and storage time had a significant effect ($p \leq 0.05$) on the total bacterial count. Following the rule of thumb described in the materials and methods section, the maximum storage time for the ATM and VAC raw peppers was 3 days and MAP raw peppers 6 days. The total number of microorganisms in the mentioned samples on the last day of storage did not exceed 4 log CFU/g, respectively: 3.98 log CFU/g; 3.99 log CFU/g; and 3.69 log CFU/g. The results on TVC microorganisms obtained in our own work correspond with the work of Yu et al. [32], who estimated the initial microbial status of sliced green peppers to be 3.36 ± 0.1 log CFU/g, and observed a sharp increase in microbial contamination to 4.88 ± 0.05 log CFU/g on day 6 of refrigerated storage (8 ± 1 °C). A study by Moreira et al. [33] also showed a significant increase in the aerobic bacterial population of green peppers during the 6-day refrigerated storage period, from 3.38 ± 0.62 log CFU/g on day 1 to 5.28 ± 0.51 log CFU/g on day 6.

The sous vide 30 min/60 °C heat treatment for all the packaging methods resulted in a significant ($p = 0.013$) reduction in the TVC bacteria counts compared to the raw peppers. On the first day of storage of the ATM- and VAC-packaged culinary products, no TVC bacteria were observed or their number was below the limit of the detection threshold (1 log CFU/g), and in the MAP-packaged peppers, the microbial count was 2.69 log CFU/g. In the test facilities, no exceedance of the safe limit of bacteria (4 log CFU/g) was observed during the entire storage period of 24 days, and the highest abundance of TVC was observed on the 18th day of storage in the MAP-packed products and their number was 3.15 log CFU/g.

For the 15 min/70 °C peppers, the packaging method and storage time did not significantly affect the microbial counts ($p = 0.102$). On the first day of storage for the 15 min/70 °C ATM and 15 min/70 °C VAC peppers, the TVC was below the limit of the detection threshold (<1 log CFU/g), while for the 15 min/70 °C MAP peppers, the number of microorganisms was 3.20 log CFU/g. Throughout the storage period of the 15 min/70 °C peppers, the number of TVC for all the packaging methods did not exceed the set limit (4 log CFU/g). The effect of the sous vide method on TVC was also checked by Rinaldi et al. [34]. They carried out microbiological analyses of steamed and sous vide (100 °C for 20 min) cooked carrots and showed that both groups of carrots showed microbial counts

that were always below 1 log colony forming units per gram (CFU/g), even after 10 days of storage at 4 °C, while the microbial status of the raw was 4.34 log CFU/g. Therefore, both heat treatments seemed to reduce the initial microbial count effectively.

In order to reduce the number of bacteria in the sous vide and VAC- and MAP-packaged products, some authors propose the use of different types of essential oils that have a positive effect on the reduction and growth of bacteria during fruit and vegetable storage, for example, *S. enterica* and *L. monocytogenes* [35–40].

Table 2. Total viable count [log CFU/g] in raw peppers and after sous vide heat treatment during storage.

Type of Packaging	Storage Day	Control	Sous Vide		
			30 min/60 °C	15 min/70 °C	8 min/80 °C
ATM	1 day	3.60 ^{a,b} ± 0.28	nd	nd	2.35 ^{a,b} ± 0.07
VAC		3.86 ^{a,b,c} ± 0.08	nd	nd	2.00 ^a ± 0.00
MAP		3.54 ^a ± 0.05	2.69 ^b ± 0.13	3.20 ^{b,c} ± 0.14	nd
ATM	3 days	3.98 ^{b,c,d} ± 0.14	nd	nd	2.84 ^{b,c,d} ± 0.76
VAC		3.99 ^{b,c,d} ± 0.06	nd	nd	2.25 ^a ± 0.07
MAP		3.61 ^{a,b} ± 0.28	2.35 ^{a,b} ± 0.21	2.80 ^{a,b,c} ± 0.31	nd
ATM	6 days	4.61 ^{e,f} ± 0.02	nd	3.01 ^{b,c} ± 0.02	3.24 ^{d,e,f} ± 0.34
VAC		4.37 ^{d,e} ± 0.02	nd	nd	nd
MAP		3.69 ^{a,b,c} ± 0.10	2.59 ^b ± 0.12	nd	2.43 ^{a,b,c} ± 0.07
ATM	9 days	nt	2.00 ^a ± 0.00	3.00 ^{a,b,c} ± 0.01	3.63 ^{e,f,g} ± 0.21
VAC		nt	2.73 ^{b,c} ± 0.47	nd	nd
MAP		4.78 ^f ± 0.28	2.60 ^b ± 0.00	3.16 ^{b,c} ± 0.23	2.96 ^{c,d} ± 0.06
ATM	12 days	nt	nd	nd	3.91 ^{g,h,i} ± 0.05
VAC		nt	nd	nd	nd
MAP		nt	nd	3.36 ^c ± 0.50	3.73 ^{f,g,h} ± 0.14
ATM	15 days	nt	2.35 ^{a,b} ± 0.07	nd	3.84 ^{g,h,i} ± 0.29
VAC		nt	nd	nd	nd
MAP		nt	nd	nd	3.65 ^{e,f,g,h} ± 0.28
ATM	18 days	nt	nd	2.65 ^{a,b} ± 0.07	4.09 ^{g,h,i} ± 0.14
VAC		nt	nd	nd	nd
MAP		nt	3.15 ^c ± 0.21	nd	3.78 ^{f,g,h} ± 0.28
ATM	21 days	nt	nd	nd	nt
VAC		nt	nd	nd	2.90 ^{c,d} ± 0.00
MAP		nt	nd	3.40 ^c ± 0.57	4.18 ^{h,i} ± 0.14
ATM	24 days	nt	2.40 ^{a,b} ± 0.14	3.16 ^{b,c} ± 0.23	nt
VAC		nt	nd	2.30 ^a ± 0.43	3.19 ^{d,e} ± 0.26
MAP		nt	nd	nd	nt
<i>p</i> -value		<0.001	0.013	0.102	<0.001

Explanations: nt—samples were not tested due to exceeding the established set limit of 4 log CFU/g; nd—not detected due to low levels below 1 log CFU/g; ^{a,b,c,...}—values in the columns marked with different lowercase letters are significantly different $p \leq 0.05$. Bold font indicates the last day of the consumer shelf life.

3.3. Evaluation of Total Enterobacteriaceae in Raw Peppers and Culinary Products During Storage

Table 3 shows the results of the Enterobacteriaceae counts for the raw peppers and sous vide cooked peppers packed in ATM, VAC, and MAP. In the raw peppers from ATM, VAC, and MAP, the total Enterobacteriaceae count on the first day of storage varied between 2.15 and 2.24 log CFU/g depending on the packaging method and no statistically significant variation was observed between the reported results. The storage time and packaging method had a

statistically significant ($p = 0.001$) effect on the Enterobacteriaceae counts in the raw peppers throughout the storage period. The Enterobacteriaceae counts on the last day of storage in the raw peppers (control) were for ATM and VAC—2 log CFU/g, while for MAP—3.80 log CFU/g. After the sous vide treatment of the peppers at 30 min/60 °C, Enterobacteriaceae were identified only in the MAP-packed products, and their counts were 2.52 log CFU/g on day 6 and 3.02 log CFU/g on day 18, and these results were not statistically significantly different ($p = 0.705$). Enterobacteriaceae were not observed in the other culinary products or their numbers were below the limit of detection threshold (1 log CFU/g). On the first day of storage of the 15 min/70 °C peppers, no Enterobacteriaceae were identified or their numbers were below the limit of detection threshold (1 log CFU/g). Regardless of the packaging methods, the Enterobacteriaceae counts throughout the storage period ranged from 2.48 log CFU/g to 3.34 log CFU/g, and the differences were not statistically significant ($p = 0.977$). After the treatment of the culinary products at 8 min/80 °C, Enterobacteriaceae were identified in the products obtained on the 15th, 18th, and 21st day of storage only for MAP packaging, and their counts were, respectively, 3.93; 3.30; and 3.91 log CFU/g. No statistically significant differences ($p = 0.805$) were observed between the MAP culinary products during storage. The assessment of the microbiological status in terms of Enterobacteriaceae contamination in the peppers and their products is not widely reported in scientific papers. However, Karikari et al. [41] found that peppers may contain unacceptable levels of Enterobacteriaceae, mainly related to inappropriate cultivation practices. They may also be contaminated with human pathogens both during harvesting and at later stages of distribution and technological processing. However, these bacteria could be reduced through good food preparation/processing practices in the food chain.

3.4. Evaluation of the Total Number of Microscopic Filamentous Fungi in Raw Peppers and Culinary Products During Storage

Table 4 shows the results of the total number of microscopic filamentous fungi (MFF) for the raw peppers and culinary peppers packed in an unaltered atmosphere (ATM), vacuum (VAC), and modified gas atmosphere (MAP). The packaging and storage time method significantly affected ($p < 0.001$) the number of MFF in the raw products. The MFF counts of the ATM and VAC raw peppers were 2.15–2.24 log CFU/g on the first day and increased to 4.43–4.44 log CFU/g on the last 6 days of storage. In contrast, on the first day of storage of the MAP raw peppers, the MFF abundance was below the limit of detection level (1 log CFU/g), and on day 9, their number increased to 3.85 log CFU/g. The number of MFF in green peppers, according to Yu et al. [32], was 3.36 ± 0.04 log CFU/g on the first day, increasing during storage to about 4.5 log CFU/g on day 6 of refrigerated storage, a value similar to our own study. Also, in the study by Zhang et al. [42], results similar to those of MFF contamination were obtained. The authors cited tested square-cut peppers packed in nitrogen on days 1, 4, and 8 of refrigerated storage at 4 °C, obtaining values of 2.33 log CFU/g, 2.52 log CFU/g, and 3.18 log CFU/g, respectively. On the other hand, slightly higher results for MFF populations were found by Moreira et al. [33], as in sliced green peppers the mentioned microorganisms showed 4.58 ± 0.22 log CFU/g on the first day of refrigerated storage at 4 °C, after which their numbers increased to 5.40 ± 0.48 log CFU/g on day 6. In all the temperature variants, the sous vide heat treatment method did not cause statistically significant changes in MFF abundance during storage, regardless of the packaging method. Irrespective of the packaging method, over the entire storage period after the cooking of the 30 min/60 °C peppers, the total MFF counts ranged from 2.15 log CFU/g to 3.52 log CFU/g, for 15 min/70 °C peppers from 2.24 log CFU/g to 3.22 log CFU/g, and for 8 min/80 °C peppers from 2.00 log CFU/g to 3.30 log CFU/g.

Table 3. Total Enterobacteriaceae [log CFU/g] in raw peppers and after sous vide heat treatment during storage.

Type of Packaging	Storage Day	Control	Sous Vide		
			30 min/60 °C	15 min/70 °C	8 min/80 °C
ATM	1 day	2.24 ^a ± 0.24	nd	nd	nd
VAC		2.15 ^a ± 0.15	nd	nd	nd
MAP		nd	nd	nd	nd
ATM	3 days	2.00 ^a ± 0.00	nd	nd	nd
VAC		2.00 ^a ± 0.00	nd	nd	nd
MAP		2.39 ^a ± 0.09	nd	nd	nd
ATM	6 days	4.41 ^b ± 0.03	nd	2.95 ^a ± 1.35	nd
VAC		4.17 ^b ± 0.09	nd	nd	nd
MAP		3.80 ^b ± 0.15	2.52 ^a ± 0.74	nd	nd
ATM	9 days	nt	nd	2.48 ^a ± 0.00	nd
VAC		nt	nd	nd	nd
MAP		3.75 ^b ± 0.75	nd	3.14 ^a ± 1.61	nd
ATM	12 days	nt	nd	nd	nd
VAC		nt	nd	nd	nd
MAP		nt	nd	3.15 ^a ± 1.62	nd
ATM	15 days	nt	nd	nd	nd
VAC		nt	nd	nd	nd
MAP		nt	nd	nd	3.93 ^a ± 0
ATM	18 days	nt	nd	nd	nd
VAC		nt	nd	nd	nd
MAP		nt	3.02 ^a ± 1.44	nd	3.30 ^a ± 1.83
ATM	21 days	nt	nd	nd	nt
VAC		nt	nd	nd	nd
MAP		nt	nd	3.34 ^a ± 1.90	3.91 ^a ± 0.00
ATM	24 days	nt	nd	nd	nt
VAC		nt	nd	nd	nd
MAP		nt	nd	nd	nt
<i>p</i> -value		<0.001	0.705	0.977	0.805

Explanations: nt—samples were not tested due to exceeding the established set limit of 4 log CFU/g; nd—not detected due to low levels below 1 log CFU/g; ^{a,b} values in the columns marked with different lowercase letters are significantly different $p \leq 0.05$.

3.5. Evaluation of Microbiological Status Based on Identified Bacteria in Pepper Samples Tested

The instrument expresses the probability of a correct identification in point form. For results between 2300 and 3000, a reliable identification of microorganisms to the species level was obtained, 2000–2299 a reliable identification to the genus level and a probable identification to the species level, results between 1700 and 1999 indicated a probable identification result to the genus level, and results below and equal to 1699 gave an unreliable identification result [43]. In this paper, results falling in the range of 2000–3000 are presented. One hundred eighty ($n = 180$) bacteria belonging to the following families were identified and correctly determined in pepper culinary products: Pseudomonadaceae 37%, Yersiniaceae 23%, Staphylococcaceae 15%, Erwiniaceae 10%, Microbacteriaceae 7%, Enterobacteriaceae 4%, and Lactobacillaceae, Brucellaceae, and Micrococcaceae 2% each. Among the correctly isolated bacteria, 75% belonged to Gram-negative (G[−]) bacteria and 25% to Gram-positive (G⁺) bacteria. In the raw peppers (control), G[−] bacteria predominated (64%), among which *Staphylococcus xylosum* 19% and *Pseudomonas rhodesiae* 17% were the most numerous groups (Figure 1). *S. xylosum*, a member of the 41 coagulase-negative species, is typically found on the skin and mucous membranes of animals, especially mammals. This species is often detected in meat products, whether fermented or dried and salted, and

is widely utilised as a starter culture in sausage production [44]. The presence of *S. xyloso* bacteria in peppers has not been reported in the scientific literature. The possible sources of these bacteria could be infections from animals or humans. *P. rhodesiae* belongs to the PGPR (plant growth-promoting rhizobacteria), which naturally influence soil health and fertility and are responsible for plant growth [45].

Table 4. Total number of microscopic filamentous fungi [log CFU/g] in raw peppers and after sous vide heat treatment during storage.

Type of Packaging	Storage Day	Control	Sous Vide		
			30 min/60 °C	15 min/70 °C	8 min/80 °C
ATM	1 day	2.24 ^a ± 0.24	nd	nd	nd
VAC		2.15 ^a ± 0.15	nd	nd	nd
MAP		nd	nd	nd	nd
ATM	3 days	2.15 ^a ± 0.10	nd	nd	nd
VAC		2.12 ^a ± 0.03	nd	2.45 ^a ± 0.64	nd
MAP		2.29 ^a ± 0.09	nd	nd	nd
ATM	6 days	4.44 ^b ± 0.09	2.15 ^a ± 0.21	2.81 ^a ± 1.14	nd
VAC		4.37 ^b ± 0.09	2.56 ^a ± 0.79	nd	nd
MAP		3.90 ^b ± 0.25	nd	nd	2.35 ^a ± 0.49
ATM	9 days	nt	nd	2.24 ^a ± 0.34	nd
VAC		nt	nd	nd	2.15 ^a ± 0.21
MAP		3.85 ^b ± 0.69	2.15 ^a ± 0.21	3.02 ^a ± 1.44	nd
ATM	12 days	nt	nd	nd	nd
VAC		nt	nd	nd	nd
MAP		nt	nd	nd	2.75 ^a ± 1.05
ATM	15 days	nt	3.39 ^a ± 1.96	2.93 ^a ± 1.31	2.68 ^a ± 0.96
VAC		nt	3.52 ^a ± 0.00	nd	2.65 ^a ± 0.92
MAP		nt	3.34 ^a ± 1.90	3.19 ^a ± 1.68	3.30 ^a ± 1.83
ATM	18 days	nt	nd	2.15 ^a ± 0.21	2.00 ^a ± 0.00
VAC		nt	nd	nd	nd
MAP		nt	3.40 ^a ± 1.98	2.72 ^a ± 1.02	3.31 ^a ± 1.85
ATM	21 days	nt	2.48 ^a ± 0.67	nd	nd.
VAC		nt	nd	nd	nd
MAP		nt	nd	3.22 ^a ± 1.73	3.09 ^a ± 1.54
ATM	24 days	nt	2.90 ^a ± 1.28	2.67 ^a ± 0.95	nt
VAC		nt	nd	nd	2.90 ^a ± 0.00
MAP		nt	nd	2.99 ^a ± 1.40	nt
<i>p</i> -value		<0.001	0.898	0.994	0.994

Explanations: nt—samples were not tested due to exceeding the established set limit of 4 log CFU/g; nd—not detected due to low levels below 1 log CFU/g; ^a, ^b—values in the columns marked with different lowercase letters are significantly different $p \leq 0.05$.

The peppers given the 30 min/60 °C heat treatment (Figure 2) contained predominantly G[−] bacteria, which accounted for 94% of all the correctly isolated bacteria. In these products, the most abundant bacteria were *Pseudomonas taetrolens* and *Serratia marcescens*, 33% each. *P. taetrolens* was selected as a plant growth-promoting rhizobacterium that is able to significantly increase phosphate uptake and phosphorus accumulation in aboveground parts (stems, petioles, and leaves) [46]. *S. marcescens* is a bacterium typically found on pepper fruits; the bacterium causes soft rot disease on peppers and has a devastating effect on the crop, and it is a disease of increasing importance worldwide causing major economic losses [47].



Figure 1. Krona chart: bacteria strains isolated from raw peppers (n = 42).

The 15 min/70 °C-treated peppers (Figure 3) were also mostly contaminated with G[−] bacteria, which accounted for 67% of all the identified bacteria; the most abundant bacteria were *Pantoea agglomerans* (26%). *P. agglomerans* is a plant-associated bacterium. No harmful effects of this bacterium on the fruit or other parts of peppers have been described in the literature, but there are scientific reports indicating that it can cause complications, bacterial soft rot of cabbage, Pepino melon blight, Ziziphus jujuba necrotic disease, brown apical necrosis of walnut, and bacterial pitting of plums. *P. agglomerans* is not an obligate infectious agent in humans, but can cause infections [48–50].

In the 8 min/80 °C products (Figure 4), G-bacteria accounted for 82% of all the bacteria, and the most abundant strain was *Serratia liquefaciens* 37%. *S. liquefaciens* promotes plant growth and yield, and in the study by Camacho-Rodriguez et al. [51], the authors observed the growth of bell pepper plants and noticed a greater number and size of fruits.

Table 5 and Table S1 (Table S1 available in Supplementary Materials) details the microbial contamination of raw peppers (control) and culinary products based on different packaging methods stored refrigerated for 24 days. The results presented in the table were analysed using three pepper packaging methods: unaltered atmosphere packaging (ATM), vacuum packaging (VAC), and modified atmosphere packaging (MAP). A total of 54 bacterial strains were isolated using the ATM method. *Microbacterium hydrocarbonoxydans* and *Microbacterium oxydans* represented the Microbacteriaceae family, detected on days 1 and 3 and day 12, respectively. The Erwiniaceae family included *Pantoea agglomerans*, detected on day 9. The Pseudomonadaceae family included multiple species: *Pseudomonas fluorescens* (days 1 and 3), *Pseudomonas libanensis* (day 9), *Pseudomonas putida* (day 3), *Pseudomonas rhodesiae* (days 3, 9, and 24), *Pseudomonas synxantha* (days 21 and 24), *Pseudomonas*

taetrolens (days 15, 18, and 24), and *Pseudomonas veronii* (day 9). The Staphylococcaceae family included *Staphylococcus haemolyticus* (day 1) and *Staphylococcus warneri* (days 1, 3, and 6). For the vacuum packaging (VAC) method, a total of nine bacterial isolates were identified; the Lactobacillaceae family was represented by *Lactiplantibacillus plantarum*, detected on day 21. The Pseudomonadaceae family included *Pseudomonas fragi* (days 9 and 12) and *Pseudomonas kilonensis* (days 1 and 3). A total of 117 isolates were isolated using the modified atmosphere packaging (MAP) method. The Enterobacteriaceae family included *Buttiauxella gaviniae* (day 9) and *Enterobacter cloacae* (days 15, 21, and 24). The Micrococcaceae family was represented by *Kocuria palustris* (day 18). The family of Microbacteriaceae included *Microbacterium oxydans* (day 18) and *Microbacterium paraoxydans* (day 12). The Brucellaceae family included *Ochrobactrum anthropi* (day 6). The family Erwiniaceae included *Pantoea agglomerans* (days 15 and 18). The family included several species: *Pseudomonas brassicacearum* (day 6).

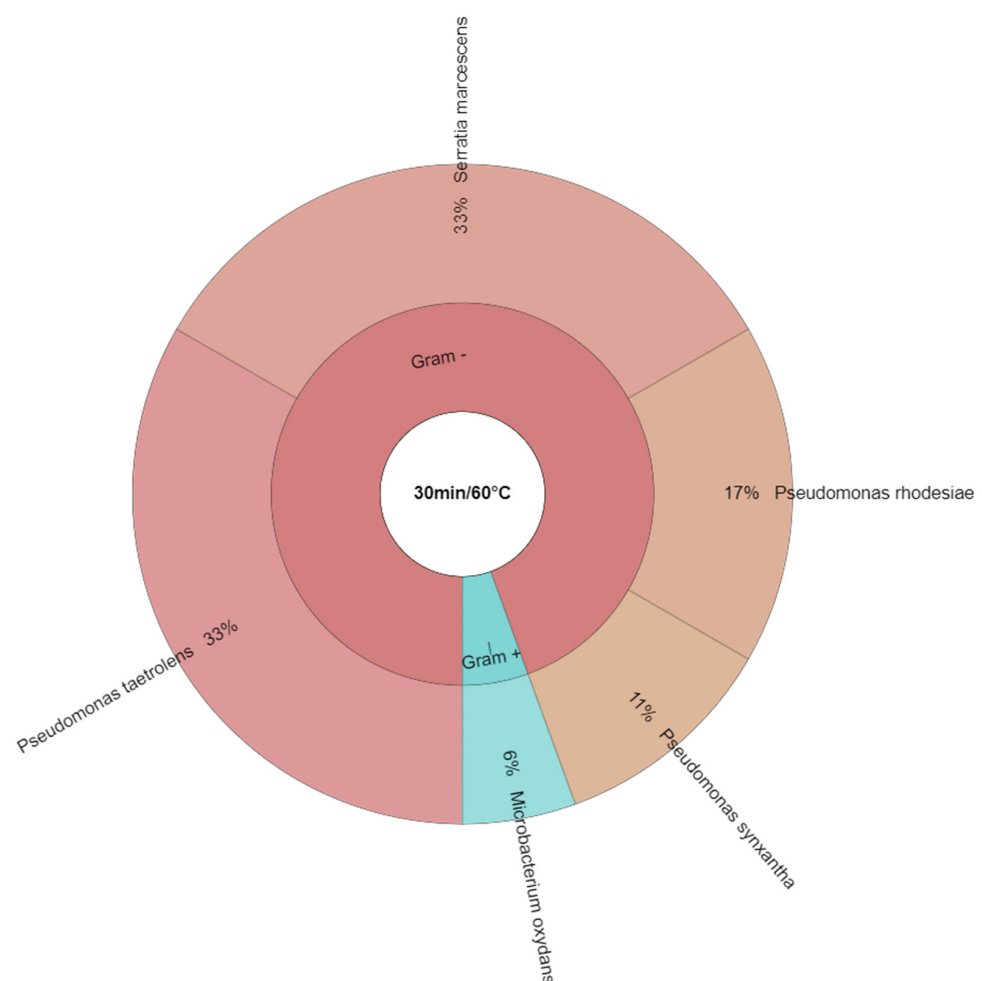


Figure 2. Krona chart: bacteria strains isolated from peppers after heat treatment 30 min/60 °C (n = 19).

Pseudomonas rhodesiae (days 15, 18, and 21), and *Pseudomonas taetrolens* (days 12, 15, and 24). The Yersiniaceae family included *Rahnella aquatilis* (days 12 and 15), *Serratia liquefaciens* (days 12, 15, and 21), and *S. marcescens* (days 1, 3, 9, and 18). The Staphylococcaceae family included *Staphylococcus haemolyticus* (day 3), *Staphylococcus pasteurii* (days 1 and 3), *Staphylococcus succinus* (day 3), *Staphylococcus warneri* (days 1 and 3), and *Staphylococcus xylosum* (days 1, 3, and 9). There are very few papers in the literature on the evaluation of the microbial status of peppers using MALDI-TOF MS technology, and it is not possible

to refer to the existing scientific literature in this area. The work of El-Nemr et al. [52] and Xu et al. [53] gives very general results of bacterial identification in fresh vegetables, including green peppers and commercially available fruits. Species-level identifications were successful for *Bacillus* spp., *Enterococcus* spp., *Escherichia* spp., *Pseudomonas* spp. *Staphylococcus* spp., *Corynebacterium* spp., *Alcaligenes* spp., and *Citrobacter* spp. Bacteria that can grow at low temperatures and survive mild heat treatment, such as *L. monocytogenes* and *E. coli*, were not found in the raw pepper samples (control) and after heat treatment in the sous vide chamber throughout the storage period. This is probably due to the high microbiological quality of the raw materials taken for testing, as well as ensuring strictly controlled temperature and asepticity during repackaging (for the ATM and MAP products). Ensuring the right conditions, i.e., the intensity of the thermal process, cooling rate, final temperature, temperature monitoring, and refrigerated storage time, as agreed by the Sous Vide Advisory Committee [54], allows food safety to be ensured. The appearance of coagulase-negative Staphylococci such as *S. haemolyticus* in the ATM and MAP samples may reflect inadequate hygiene and cross-contamination, as this bacterium is a normal skin commensal, but high contamination with enterotoxigenic staphylococci may be a potential indicator of the quality and evaluation of food poisoning risk [55].



Figure 3. Krona chart: bacteria strains isolated from peppers after heat treatment 15 min/70 °C (n = 70).



Figure 4. Krona chart: bacteria strains isolated from peppers after heat treatment 8 min/80 °C (n = 49).

Studies have shown that the use of sous vide heat treatment combined with appropriate packaging methods (VAC and MAP) significantly extends the shelf life of red peppers. This means that producers and distributors can plan for longer storage periods, reducing losses due to food spoilage. The choice of the appropriate food packaging method can be tailored to the specific needs of distribution and storage [56,57]. Sous vide processing effectively reduces the number of TVC, Enterobacteriaceae, and the number of microscopic filamentous fungi, which ensures a higher microbiological quality of the product. This makes peppers safer for consumers and significantly reduces the risk of bacterial contamination. This can influence marketing strategies, emphasising the safety and quality of the product. However, further research is needed on other vegetables to better understand the impact of packaging methods and gas atmosphere composition. Each vegetable has unique structural properties, nutritional value, and a profile of bioactive substances that can act as biopreservatives. In the future, different gas combinations for MAP should be investigated to determine the most effective mixtures for extending shelf life and maintaining the quality of different vegetables, testing CO₂, N₂, and O₂ in various proportions.

With the possibilities offered by sous vide technology, manufacturers can introduce new products to the market, such as ready-to-eat meals from peppers and other vegetables. This allows them to diversify their offer and attract new customer segments. Companies implementing modern processing and packaging technologies can gain a competitive advantage in the market. Innovative approaches to food production can attract customers looking for modern and safe products [58].

Table 5. Isolated species from samples depending on the packaging method.

Sposób Pakowania	Family	Species	Number of Isolates	Day									
				1	3	6	9	12	15	18	21	24	
ATM	Microbacteriaceae	<i>Microbacterium hydrocarbonoxydans</i>	4	+	+								
	Microbacteriaceae	<i>Microbacterium oxydans</i>	2					+					
	Erwiniaceae	<i>Pantoea agglomerans</i>	9					+					
	Pseudomonadaceae	<i>Pseudomonas fluorescens</i>	5	+	+								
	Pseudomonadaceae	<i>Pseudomonas libanensis</i>	1					+					
	Pseudomonadaceae	<i>Pseudomonas putida</i>	3		+								
	Pseudomonadaceae	<i>Pseudomonas rhodesiae</i>	10		+		+						+
	Pseudomonadaceae	<i>Pseudomonas synxantha</i>	5									+	+
	Pseudomonadaceae	<i>Pseudomonas taetrolens</i>	6							+	+		+
	Pseudomonadaceae	<i>Pseudomonas veronii</i>	3				+						
	Staphylococcaceae	<i>Staphylococcus haemolyticus</i>	1	+									
	Staphylococcaceae	<i>Staphylococcus warneri</i>	5	+	+	+							
Total ATM			54										
VAC	Lactobacillaceae	<i>Lactobacillus plantarum</i>	3									+	
	Pseudomonadaceae	<i>Pseudomonas fragi</i>	2					+	+				
	Pseudomonadaceae	<i>Pseudomonas kilonensis</i>	4	+	+								
Total VAC			9										
MAP	Enterobacteriaceae	<i>Buttiauxella gaviniae</i>	1				+						
	Enterobacteriaceae	<i>Enterobacter cloacae</i>	5							+		+	+
	Micrococcaceae	<i>Kocuria palustris</i>	3								+		
	Microbacteriaceae	<i>Microbacterium oxydans</i>	1								+		
	Microbacteriaceae	<i>Microbacterium paraoxydans</i>	5					+					
	Brucellaceae	<i>Ochrobactrum anthropi</i>	3			+							
	Erwiniaceae	<i>Pantoea agglomerans</i>	10							+	+		
	Pseudomonadaceae	<i>Pseudomonas brassicacearum</i>	1			+							
	Pseudomonadaceae	<i>Pseudomonas lundensis</i>	1			+							
	Pseudomonadaceae	<i>Pseudomonas rhodesiae</i>	16							+	+	+	
	Pseudomonadaceae	<i>Pseudomonas taetrolens</i>	6						+	+			+
	Yersiniaceae	<i>Rahnella aquatilis</i>	5					+	+				
	Yersiniaceae	<i>Serratia liquefaciens</i>	27					+	+			+	
	Yersiniaceae	<i>Serratia marcescens</i>	9	+	+		+				+		
	Staphylococcaceae	<i>Staphylococcus haemolyticus</i>	2		+								
	Staphylococcaceae	<i>Staphylococcus pasteurii</i>	5	+	+								
	Staphylococcaceae	<i>Staphylococcus succinus</i>	3		+								
	Staphylococcaceae	<i>Staphylococcus warneri</i>	6	+	+								
	Staphylococcaceae	<i>Staphylococcus xylosus</i>	8	+	+		+						
Total MAP			117										
Total isolates			180										

Explanation: “+”—occurrence of the bacterial isolate on the tested day.

4. Conclusions

The study showed that the sous vide heat treatment of red peppers compared to the control has an effect on the decrease in pH (significant for VAC and MAP) and the number of TVC, Enterobacteriaceae, and MFF, the latter two groups belonging to the scope of disclosure (1 log CFU/g). The use of combined methods and the final result of the culinary products effectively extend the shelf life of sous vide peppers. Compared to the raw peppers, the shelf life of the culinary products increased from 3 to 6 days to 15–24 days depending on the packaging method. For the 30 min/60 °C and 15 min/70 °C treatments, all the packaging methods allowed the product to remain health-safe (TVC < 4 log CFU/g) for 24 days, while for the 8 min/80 °C treatment, VAC packaging was the most effective and also protected the product for 24 days. Based on the analyses, it was shown that the best results were obtained for the peppers treated at 30 min/60 °C and 15 min/70 °C, where TVC, Enterobacteriaceae, and MFF were the lowest and did not exceed the safe limit of 4 log CFU/g for the entire storage period (24 days). Bacterial identification using MALDI-TOF MS identified the predominant bacterial families in raw and post-cooking peppers. The dominant families were Pseudomonadaceae, Yersiniaceae, and Staphylococcaceae. The sous vide heat treatment conditions resulted in differences in the percentage of bacterial families in the culinary products. The proportions in the participation of Gram+ and Gram- also changed.

At this stage of the research, only aerobic bacteria have been identified. In the future, the identification of bacteria will be extended to include anaerobic bacteria, which may be present in the packaging methods used in this research.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app15031303/s1>, Table S1. Isolated species from samples according to treatment method, packaging method and day of storage.

Author Contributions: Conceptualisation, P.H., G.J. and K.P.; methodology, P.H., G.J. and M.K.; software, P.H. and N.S.; validation, G.J., K.P. and M.K.; formal analysis, P.H. and N.S.; investigation, P.H. and G.J.; resources, P.H. and G.J.; data curation, P.H. and G.J.; writing—original draft preparation, P.H., G.J. and K.P.; writing—review and editing, P.H., G.J. and K.P.; visualisation, P.H. and N.S.; supervision, G.J. and K.P.; funding acquisition K.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

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

References

1. Jimenez-García, S.N.; Garcia-Mier, L.; Ramirez-Gomez, X.S.; Guevara-Gonzalez, R.G.; Aguirre-Becerra, H.; Escobar-Ortiz, A.; Contreras-Medina, L.M.; Garcia-Trejo, J.F.; Vazquez-Cruz, M.A.; Feregrino-Perez, A.A. Characterization of the Key Compounds of Bell Pepper by Spectrophotometry and Gas Chromatography on the Effects of Induced Stress on the Concentration of Secondary Metabolite. *Molecules* **2023**, *28*, 3830. [CrossRef] [PubMed]
2. Grzesińska, W.; Tomaszewska, M. Technologia sous-vide a jakość potraw. Tajniki sous-vide. *Przegląd Gastrologiczny* **2013**, *3*, 6–11.
3. Holdsworth, S.D.; Simpson, R. Optimization of Thermal Food Processing. In *Thermal Processing of Packaged Foods*; Food Engineering Series; Springer: Cham, Switzerland, 2016. [CrossRef]
4. Michalak-Majewska, M.; Stanikowski, P.; Gustaw, W.; Sławińska, A.; Radzki, W.; Skrzypczak, K.; Jabłońska-Ryś, E. Technologia sous-vide—Innowacyjny sposób obróbki cieplnej żywności. *ŻYWNOSĆ Nauka Technol. Jakość* **2018**, *3*, 34–44. [CrossRef]

5. Łepecka, A.; Zielińska, D.; Szymański, P.; Buras, I.; Kołożyn-Krajewska, D. Assessment of the Microbiological Quality of Ready-to-Eat Salads—Are There Any Reasons for Concern about Public Health? *Int. J. Environ. Res. Public Health* **2022**, *19*, 1582. [\[CrossRef\]](#)
6. Chaki, M.; Ruiz, C.C.J.; Barroso, J.B.; Corpas, F.J.; Palma, J.M. Ripening of pepper (*Capsicum annuum*) fruit is characterized by an enhancement of protein tyrosine nitration. *Ann. Bot.* **2015**, *116*, 637–647. [\[CrossRef\]](#)
7. Palma, J.M.; Sevilla, F.; Jiménez, A.; Corpas, F.J.; Camejo, D.M. Physiology of pepper fruit and the metabolism of antioxidants: Chloroplasts, mitochondria and peroxisomes. *Ann. Bot.* **2015**, *116*, 627–636. [\[CrossRef\]](#)
8. Rybak, K.; Wiktor, A.; Witrowa-Rajchert, D.; Parniak, O.; Nowacka, M. The Effect of Traditional and Non-Thermal Treatments on the Bioactive Compounds and Sugars Content of Red Bell Pepper. *Molecules* **2020**, *25*, 4287. [\[CrossRef\]](#)
9. Duma, M. Chemical composition of Tomatoes depending on the stage of ripening. *Chemine Technol.* **2015**, *1*, 24–28. [\[CrossRef\]](#)
10. Tudor-Radu, M.; Vijan, L.E.; Tudor-Radu, C.M.; Tita, I.; Sima, R.; Mitrea, R. Assessment of Ascorbic Acid, Polyphenols, Flavonoids, Antocyanins and Carotenoids Content in Tomato Fruits. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2016**, *44*, 477–483. [\[CrossRef\]](#)
11. Kumari, S.; Anokhe, A.; Kumar, R. Nutritional quality and health benefits of solanaceous vegetables. *Progress. Res. Int. J.* **2017**, *12*, 1942–1945.
12. Domínguez, R.; Bohrer, B.; Pateiro, M.; Sichert Munekata, P.E.; Lorenzo, J.M. Chapter 4—Packaging Systems. In *Sustainable Production Technology in Food*; Lorenzo, J.M., Munekata, P.E.S., Barba, F.J., Eds.; Academic Press: Cambridge, MA, USA, 2021; pp. 49–69.
13. Zavadlav, S.; Blažić, M.; Vignatti, C.; Fenoglio, C.; Piagentini, A.M.; Pirovani, M.E.; Perotti, C.M.; Kovačević, D.B.; Putnik, P. Sous-Vide as a Technique for Preparing Healthy and High-Quality Vegetable and Seafood Products. *Foods* **2020**, *9*, 1537. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Baldwin, D.E. Sous Vide Cooking: A Review. *Int. J. Gastron. Food Sci.* **2012**, *1*, 15–30. [\[CrossRef\]](#)
15. Stringer, S.C.; Metris, A. Predicting bacterial behaviour in sous vide food. *Int. J. Gastron. Food Sci.* **2018**, *13*, 117–128. [\[CrossRef\]](#)
16. Singh, P.; Sultan, Z.; Pandey, V.K.; Singh, R. Sous vide processing for food quality enhancement: A review. *Food Humanit.* **2023**, *1*, 543–552. [\[CrossRef\]](#)
17. Iborra-Bernad, C.; García-Segovia, P.; Martínez-Monzó, J. Physico-Chemical and Structural Characteristics of Vegetables Cooked Under Sous-Vide, Cook-Vide, and Conventional Boiling. *J. Food Sci.* **2015**, *80*, 1725–1734. [\[CrossRef\]](#)
18. Stankov, S.; Fidan, H.; Rusev, R.; Baeva, M. Low-temperature cooking method “sous vide” in the restaurant industry: A review. *Food Sci. Appl. Biotechnol.* **2020**, *3*, 92–102. [\[CrossRef\]](#)
19. Onyeaka, D.H.; Nwaizu, C.; Ekaette, I. Mathematical modeling for thermally treated vacuum-packaged foods: A review on sous vide processing. *Trends Food Sci. Technol.* **2022**, *126*, 73–85. [\[CrossRef\]](#)
20. Souza, A.G.; Ferreira, R.R.; Paula, L.C.; Mitra, S.K.; Rosa, D.S. Starch-based films enriched with nanocellulose-stabilized Pickering emulsions containing different essential oils for possible applications in food packaging. *Food Packag. Shelf Life* **2021**, *27*, 100615. [\[CrossRef\]](#)
21. Chen, L.; Zhang, H.; Li, S.; Li, Y.; Zhao, Y.; Zeng, Q.; Liu, H.; Wang, J.J. Novel antimicrobial polyvinyl alcohol film by incorporating β -cyclodextrin/berberine inclusion complex preserving the storage quality of salmon fillets. *Food Packag. Shelf Life* **2024**, *46*, 101354. [\[CrossRef\]](#)
22. ISO 750:1998; Fruit and Vegetable Products—Determination of Titratable Acidity. International Organization for Standardization: Geneva, Switzerland, 1998.
23. ISO 11133:2014; Microbiology of Food, Animal Feed and Water—Preparation, Production, Storage and Performance Testing of Culture Media. International Organization for Standardization: Geneva, Switzerland, 2014.
24. ISO 9308-1:2014; Water Quality—Enumeration of *Escherichia coli* and Coliform bacteria. International Organization for Standardization: Geneva, Switzerland, 2014.
25. ISO 16212:2017; Cosmetics—Microbiology—Enumeration of Yeast and Mould. International Organization for Standardization: Geneva, Switzerland, 2017.
26. ISO 4833-1:2013; Microbiology of the Food Chain—Horizontal Method for the Enumeration of Microorganisms—Part 1: Colony Count at 30 °C by the Plate Count Technique. International Organization for Standardization: Geneva, Switzerland, 2013.
27. Kačániová, M.; Galovičová, L.; Borotová, P.; Vukovic, N.L.; Vukic, M.; Kunová, S.; Hanus, P.; Bakay, L.; Zagrobelna, E.; Kluz, M. Assessment of *Ocimum basilicum* Essential Oil Anti-Insect Activity and Antimicrobial Protection in Fruit and Vegetable Quality. *Plants* **2021**, *11*, 1030. [\[CrossRef\]](#)
28. Dimassi, O.; Fakih, Y.; Sharif-Askari, E.; Rached, M.; Akiki, R. Drying dynamics, Physicochemical properties and Sensory Analysis of Sweet Red Pepper Paste. *Int. J. Hort. Agric. Food Sci.* **2019**, *3*, 216–223. [\[CrossRef\]](#)
29. Rodoni, L.; Hasperué, J.; Ortiz, C.; Lemoine, M.; Concellón, A.; Vicente, A. Combined use of mild heat treatment and refrigeration to extend the postharvest life of organic pepper sticks, as affected by fruit maturity stage. *Postharvest Biol. Technol.* **2016**, *117*, 168–176. [\[CrossRef\]](#)

30. Bozkurt, H.; Erkmen, O. Effects of production techniques on the quality of hot pepper paste. *J. Food Eng.* **2004**, *64*, 173–178. [\[CrossRef\]](#)
31. Dos Reis, L.C.R.; De Oliveira, V.R.; Hagen, M.E.K.; Jablonski, A.; Flôres, S.H.; De Oliveira Rios, A. Effect of cooking on the concentration of bioactive compounds in broccoli (*Brassica oleracea* var. Avenger) and cauliflower (*Brassica oleracea* var. Alphina F1) grown in an organic system. *Food Chem.* **2015**, *172*, 770–777. [\[CrossRef\]](#)
32. Yu, Y.; Qiao, J.; Zhang, S.; Li, H.; Huang, S.; Qin, Y. Synergistic Utilization of ϵ -Polylysine and p-Coumaric Acid as Natural Preservatives for Enhancing the Shelf Life of Fresh-Cut Green Bell Peppers. *J. Food Biochem.* **2023**, *1*, 5435011. [\[CrossRef\]](#)
33. Moreira, J.; Mera, E.; Singh Chhetri, V.; King, J.; Gentimis, T.; Adhikari, A. Effect of storage temperature and produce type on the survival or growth of *Listeria monocytogenes* on peeled rinds and fresh-cut produce. *Front. Microbiol.* **2023**, *14*, 1151819. [\[CrossRef\]](#)
34. Rinaldi, M.; Dall'Asta, C.; Meli, F.; Morini, E.; Pellegrini, N.; Gatti, M.; Chiavaro, E. Physicochemical and Microbiological Quality of Sous-Vide-Processed Carrots and Brussels Sprouts. *Food Bioprocess Technol.* **2012**, *6*, 3076–3087. [\[CrossRef\]](#)
35. Guo, J.; Gao, Z.; Li, G.; Fu, F.; Liang, Z.; Zhu, H.; Shan, Y. Antimicrobial and antibiofilm efficacy and mechanism of essential oil from *Citrus Changshan-huyou* Y. B. Chang against *Listeria monocytogenes*. *Food Control.* **2019**, *105*, 256–264. [\[CrossRef\]](#)
36. Schneider, G.; Steinbach, A.; Putics, Á.; Solti-Hodován, Á.; Palkovics, T. Potential of Essential Oils in the Control of *Listeria monocytogenes*. *Microorganisms* **2023**, *11*, 1364. [\[CrossRef\]](#)
37. Čmiková, N.D.; Vukic, M.L.; Vukovic, N.; Verešová, A.; Bianchi, A.; Garzoli, S.; Ben Saad, R.; Ben Hsouna, A.; Ban, Z.; Kačániová, M. Phytochemical investigation, evaluation of the biological activities and preservative effect of the essential oil of *Juniperus communis* L. Dried berries on the vacuum-packed carrot after the application of *Salmonella enterica*. *Sci. Hort.* **2024**, *336*, 113442. [\[CrossRef\]](#)
38. Kačániová, M.; Čmiková, N.; Vukovic, N.L.; Verešová, A.; Bianchi, A.; Garzoli, S.; Ben Saad, R.; Ben Hsouna, A.; Ban, Z.; Vukic, M.D. *Citrus limon* Essential Oil: Chemical Composition and Selected Biological Properties Focusing on the Antimicrobial (In Vitro, In Situ), Antibiofilm, Insecticidal Activity and Preservative Effect against *Salmonella enterica* Inoculated in Carrot. *Plants* **2024**, *13*, 524. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Kačániová, M.; Vukovic, N.L.; Čmiková, N.; Bianchi, A.; Garzoli, S.; Ben Saad, R.; Ben Hsouna, A.; Elizondo-Luévano, J.H.; Said-Al Ahl, H.A.H.; Hikal, W.M.; et al. Biological Activity and Phytochemical Characteristics of Star Anise (*Illicium verum*) Essential Oil and Its Anti-*Salmonella* Activity on Sous Vide Pumpkin Model. *Foods* **2024**, *13*, 1505. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Verešová, A.; Vukic, M.D.; Vukovic, N.L.; Terentjeva, M.; Ban, Z.; Li, L.; Bianchi, A.; Kollár, J.; Ben Saad, R.; Ben Hsouna, A.; et al. Chemical Composition, Biological Activity, and Application of *Rosa damascena* Essential Oil as an Antimicrobial Agent in Minimally Processed Eggplant Inoculated with *Salmonella enterica*. *Foods* **2024**, *13*, 3579. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Karikari, A.B.; Kpordze, S.W.; Yamik, D.Y.; Saba, C.K. Ready-to-Eat Food as Sources of Extended-Spectrum β -Lactamase-Producing *Salmonella* and *E. Coli* in Tamale, Ghana. *Front. Trop. Dis.* **2022**, *3*, 834048. [\[CrossRef\]](#)
42. Zhang, Y.; Sun, D.W. Comparative Study of Sous Vide and Traditional Blanching on the Quality of Red Peppers. *J. Food Process. Preserv.* **2019**, *43*, e13796.
43. Augustyńska-Prejsnar, A.; Kačániová, M.; Ormian, M.; Topczewska, J.; Sokołowicz, Z.; Hanus, P. Quality Assessment of Minced Poultry Products Including Black Fermented Garlic. *Foods* **2023**, *13*, 70. [\[CrossRef\]](#)
44. Leroy, S.; Vermassen, A.; Ras, G.; Talon, R. Insight into the Genome of *Staphylococcus xylosus*, a Ubiquitous Species Well Adapted to Meat Products. *Microorganisms* **2017**, *5*, 52. [\[CrossRef\]](#)
45. Ye, S.; Yan, R.; Li, X.; Lin, Y.; Yang, Z.; Ma, Y.; Ding, Z. Biocontrol potential of *Pseudomonas rhodesiae* GC-7 against the root-knot nematode *Meloidogyne graminicola* through both antagonistic effects and induced plant resistance. *Front. Microbiol.* **2022**, *13*, 1025727. [\[CrossRef\]](#)
46. Ghorehshizadeh, S.; Calvo-Peña, C.; Ruiz-Muñoz, M.; Otero-Suárez, R.; Coque, J.J.R.; Cobos, R. *Pseudomonas taetrolens* ULE-PH5 and *Pseudomonas* sp. ULE-PH6 Isolated from the Hop Rhizosphere Increase Phosphate Assimilation by the Plant. *Plants* **2024**, *13*, 402. [\[CrossRef\]](#)
47. Gillis, A.; Rodríguez, M.; Santana, M.A. *Serratia marcescens* associated with bell pepper (*Capsicum annuum* L.) soft-rot disease under greenhouse conditions. *Eur. J. Plant Pathol.* **2014**, *138*, 1–8. [\[CrossRef\]](#)
48. Dutkiewicz, J.; Mackiewicz, B.; Lemieszek, K.M.; Golec, M.; Milanowski, J. *Pantoea agglomerans*: A mysterious bacterium of evil and good. Part III. Deleterious effects: Infections of humans, animals and plants. *Ann. Agric. Environ. Med.* **2016**, *23*, 197–205. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Shu, R.; Yin, X.; Long, Y.; Yuan, J.; Zhou, H. Detection and Control of *Pantoea agglomerans* Causing Plum Bacterial Shot-Hole Disease by Loop-Mediated Isothermal Amplification Technique. *Front. Microbiol.* **2022**, *13*, 896567. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Dutkiewicz, J.; Mackiewicz, B.; Lemieszek, M.K.; Golec, M.; Milanowski, J. *Pantoea agglomerans*: A mysterious bacterium of evil and good. Part IV. Beneficial effects. *Ann. Agric. Environ. Med.* **2016**, *23*, 206–222. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Camacho-Rodríguez, M.; Almaraz-Suárez, J.J.; Vázquez-Vázquez, C.; Angulo-Castro, A.; Ríos-Vega, M.E.; González-Mancilla, A. Effect of plant growth-promoting rhizobacteria on the development and yield of jalapeño pepper. *Mex. J. Agric. Sci.* **2022**, *13*, 185–196. [\[CrossRef\]](#)

52. El-Nemr, I.M.; Mushtaha, M.; Sundararaju, S.; Fontejon, C.; Suleiman, M.; Tang, P.; Hasan, M.R. Application of MALDI Biotyper System for Rapid Identification of Bacteria Isolated from a Fresh Produce Market. *Curr. Microbiol.* **2019**, *76*, 290–296. [\[CrossRef\]](#)
53. Xu, X.; Liu, G.; Huang, X.; Li, L.; Lin, H.; Xu, D. MALDI-TOF MS-Based identification of bacteria and a survey of fresh vegetables with pathogenic bacteria in Beijing, China. *Food Biosci.* **2021**, *41*, 100746. [\[CrossRef\]](#)
54. SVAC Sous Vide Advisory Committee. *Code of Practice for Sous Vide Catering Systems*; SVAC: Tetbury, UK, 1991.
55. Sebasti  , C.; Soriano, J.M.; Iranzo, M.; Rico, H. Microbiological quality of sous vide cook–chill preserved food at different shelf life. *J. Food Process. Preserv.* **2010**, *34*, 964–974. [\[CrossRef\]](#)
56. Lu, S.H.; Raj, R.; Mahajan, A.; Jha, A.; Verma, P.; Lan, H.P.; Singh, S. Rethinking and redesigning storage, packaging and distribution practices for food supply chain. *Br. Food J.* **2024**; *ahead-of-print*. [\[CrossRef\]](#)
57. D  rnyei, K.R.; Krauter, V.; Weinrich, R.; Incarnato, L.; Karlovits, I.; Colelli, G.; Chrysoschou, P.; Fenech, M.C.; Pettersen, M.K.; Arranz, E.; et al. Sustainable food packaging: An updated definition following a holistic approach. *Front. Sustain. Food Syst.* **2023**, *7*, 1119052. [\[CrossRef\]](#)
58. Venkatesan, U.; Muniyan, R. Review on the extension of shelf life for fruits and vegetables using natural preservatives. *Food Sci. Biotechnol.* **2024**, *33*, 2477–2496. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.