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Scientific Research

Investigating the effect of ohmic and water bath heating on surviving of *Escherichia coli*

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ABSTRACT

Carrot juice has a very short shelf-life after production. Pasteurization is a suitable way to preserve and commercialize this product. In this study, an ohmic device was used as a heat source and *Escherichia coli* was chosen as an indicator of the effectiveness of bacterial inactivation for pasteurization. Fresh carrot juice was subjected to 3 temperature levels of 48, 55, and 62°C and 6 time levels of 0, 30, 60, 90, 120, and 150 seconds at a constant voltage of 100 volts under thermal processing and the number of surviving bacteria was investigated. . Conventional hot water bath pasteurization was also performed and compared with the ohmic method, which proved the greater efficiency of the ohmic method. Ohmic pasteurization not only prevents waste of time and energy but also leads to the inactivation of bacterial vegetative forms in a very short time at high speed. The changes in surviving bacteria with increasing temperature and time were significant at the 1% probability level using a completely randomized method. At 62°C, the best result for *Escherichia coli* was obtained.

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1-Introduction

Carrot is a vegetable belonging to the *Apiaceae* family, classified as *Daucus carota L*. Carrot is an edible root or tuber vegetable that can be eaten raw, grated in salads, or used in soups and stews. The provinces of East Azerbaijan, Khuzestan, Isfahan, and Zanjan have the largest portion in the country's carrot production. Carrot contains high amounts of beta-carotene, a precursor of vitamin A, and contains various minerals such as iron, copper, magnesium, phosphorus, and vitamins such as B and C groups [1]. Generally, with the growing popularity of ready-to-eat foods in many Asian countries, there has been an increasing demand for high-quality processed vegetables and fruits, and this trend is expected to continue in all developing countries worldwide over the next decade [2]. Carrot juice is a highly nutritious drink rich in beta-carotene and antioxidants. Additionally, carrot roots produce the phytoalexin 6-methoxymellein, which inhibits the growth of several fungi, yeasts, and molds and show anti-Listeria effects. However, the high pH of carrot juice (6.4 to 6.8) still challenges the product's shelf life, as undesirable microorganisms are highly resistant at these pH levels [3].

Among all possible causes of food spoilage, the spread of microorganisms in food is of particular importance in the food industry. In addition to food waste caused by discarding contaminated products, some microorganisms can also cause public health concerns. These problems are related to the pathologies caused by the microorganisms themselves or the toxins produced by some species. Therefore, inactivating microorganisms to an acceptable number is one of the most important concerns in food processing [4]. Traditionally, fruit juice acidity was considered an important barrier to the growth of foodborne pathogens. However, many studies on the prevalence of foodborne diseases involving fruit juice consumption have shown that acidic fruit juices can carry human pathogens. *Escherichia coli* O157:H7 can survive well in acidic foods and beverages as it tolerates some organic acids and has acid adaptation capabilities [5]. *Escherichia coli* O157:H7, a type of Gram-negative pathogen, is a subset of enterohemorrhagic *E. coli* and is listed by the United States Centers for Disease Control and Prevention as one of the top five pathogens leading to hospitalization. Therefore, effective inactivation of *Escherichia coli* O157:H7 in food is important to prevent disease outbreaks and ensure food safety and human health [6].

Thermal processes, including pasteurization, have been used as a thermal processing method to inactivate pathogenic and spoilage microorganisms to ensure the safety and extend the shelf life of various foods. Carrot juice, as a fresh and liquid product, is susceptible to the growth of pathogenic microorganisms that can cause gastrointestinal and toxic infections. Some of these microorganisms include *Escherichia coli*, Salmonella, Listeria, and Clostridium botulinum. Pasteurization of carrot juice can kill or weaken these bacteria and prevent the risk of foodborne diseases. Additionally, pasteurization can extend the shelf life of carrot juice and reduce the need for preservatives and additives [7]. However, new thermal technologies, compared to traditional thermal treatments, can better preserve the nutrients, texture, color, and flavor of foods. Therefore, the development of new thermal processing technologies has attracted special attention in recent decades. Ohmic heating can be used as an effective thermal process for continuously heating food products by passing electrical currents through food matrices. Thus, ohmic heating can bypass the limitations of conventional pasteurization using electricity [8]. Ohmic heating technology is based on passing alternating electrical currents through a food material with the primary aim of heating it [9]. The most important advantage associated with this process is the ability to heat materials quickly and uniformly, including particulate products, and for this reason, products treated with ohmic heating will have higher quality

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compared to conventionally processed products [10]. Its advantages over conventional heating include preserving food color and value, high heating speed, volumetric heating, no temperature gradient, environmental friendliness, and very high efficiency and yield [11]. This study examined the use of ohmic heating and its effects on inactivating *Escherichia coli* in carrot juice.

2- Materials and Methods

2.1. Materials

We purchased carrot samples, from the family *Apiaceae* and the genus *Daucus carota*, from a garden in Isfahan and transferred them to the laboratory. High-purity chemicals were obtained from Merck, Germany.

2.2. Methods

2.2.1. Sample Preparation

Fresh carrot samples were cleaned, peeled, and washed thoroughly with distilled water, then juiced using a juicer [12]. The pH was then adjusted, and the juice was autoclaved (121°C, 1.2 bar pressure for 20 minutes) in 250 mL heat-resistant glass bottles to ensure they were free of any microorganisms. The samples were stored in a refrigerator (4°C) until testing.

2.2.2. Culture Media and Dilution Serums

In this study, three culture media (BHI, EMB, and PCA) and a physiological serum diluent were used for the main experiment, which involved counting the remaining colonies. Additionally, three culture media (SIM, MR-VP, and Simmons Citrate) were used for the IMViC confirmation test, and NB medium with 1% glucose, lactose, and sucrose along with phenol red indicator was used for the sugar fermentation test. Each medium was prepared according to the manufacturer's instructions, sterilized by autoclaving at 121°C, 1.2 bar pressure for 20 minutes, and stored in sterile packs in a refrigerator (4°C) until testing.

To prepare the physiological serums used for dilution, we weighed 8.5 grams of sodium chloride (accuracy 0.0001) and dissolved it in distilled water, and then adjusted the volume to one liter. Using a pipette, 9 mL of the serum was transferred into test tubes, sealed with cotton, grouped in rows of ten, and sterilized by autoclaving at 121°C, 1.2 bar pressure for 20 minutes. The sterile packs were stored in a refrigerator (4°C) until use [13].

2.2.3. *Escherichia coli* Sample

The *Escherichia coli* strain (PTCC 1399) was purchased from the Iranian Research Organization for Science and Technology. To fully activate the strain, it was first placed in BHI medium and incubated at 37°C for 48 hours in an incubator (Pars-Teb Novin, Iran). This process was repeated three times with new bacterial passages from the previous culture every 48 hours. After observing the sufficient turbidity, a sterile swab was used to transfer the sample to EMB medium using the streak plate method. After incubation at 40°C and the formation of a green sheen, the *Escherichia coli* sample was ready. The plate could be stored in a refrigerator for about three weeks, and IMViC and sugar fermentation tests were performed to verify bacterial purity. The pure *Escherichia coli* was used to prepare the McFarland standard [14].

To prepare the McFarland standard for the final sample inoculation with carrot juice, a spectrophotometer (UNICO S2100, USA) was used. Several single colonies from the EMB medium were isolated and incubated in BHI medium for 24 hours at 37°C to create an overnight culture. This overnight culture was used to prepare the McFarland standard. In a completely sterile environment, the absorbance was set to 625 nm, and the spectrophotometer was calibrated with a blank BHI medium to zero. The absorbance of the inoculated sample

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was adjusted to 0.09, which corresponds to a bacterial count of approximately 1.5×10^6 CFU/mL [15].

2.2.4. IMViC Confirmation Test for *Escherichia coli*

The Indole test and citrate utilization test assess the ability of the bacteria to produce specific enzymes. On the other hand, the Methyl red test and Voges-Proskauer test identify the final metabolic products produced by the bacteria. Thus, the type and genus of the bacteria are determined based on the enzymes, required nutrients, and metabolic products they produce [16].

2.2.5. Sugar Fermentation Confirmation Test for *Escherichia coli*

This includes three sugar fermentation tests for glucose, lactose, and sucrose. These tests were conducted in liquid nutrient broth by adding 1% of each sugar, and the presence or absence of *Escherichia coli* is confirmed by adding phenol red indicator. The presence of *Escherichia coli* was indicated by a color change of phenol red from red to yellow in all three sugars [15].

2.2.6. pH Adjustment

The pasteurization range for foods is for those with a pH less than 4.6. Since the carrot juice used in this study had an initial pH of 6.6, which is not within the pasteurization range, we adjusted the pH to the target level of 4.4 using food-grade acids within permissible limits [17]. For this purpose, the carrot juice sample is placed in a container, and a pH meter electrode is inserted. A mixture of phosphoric acid and glucono-delta-lactone (ratio 1:3) was slowly added to the sample and stirred. Due to significant changes, this mixture was used to pH 5, and the remaining adjustment was done with 3M phosphoric acid solution. The amount of acid used was calculated according to Henderson–Hasselbalch [۱۸], equation (1):

$$pH = pk_a + log_{۱۰}\left(\frac{[A^-]}{[HA]}\right) \quad (۱)$$

Where K_a is acid dissociation constant, $[A^-]$ is concentration of the conjugate base of the acid, and [HA] stands for concentration of chemical species HA, [۱۹].

2.2.7. Hot Water Bath Pasteurization

First, the water bath thermometer reached the target temperatures (48, 55, 62°C). A sterile pack with 250 mL of inoculated sample was placed in the bath, and the sample temperature was monitored with a thermometer until it reached the target temperature. The time taken for the sample to reach the target temperature from 25°C is termed the come-up time, which is important in thermal calculations and was measured in this study. The survival of bacteria in the sample was examined at 48, 55, and 62°C at five-time intervals: 0, 30, 60, 90, 120, and 150 seconds.

2.2.8. Ohmic Pasteurization

In this method, samples were placed in a specially designed cell that has been previously sterilized by autoclaving and heated to 48, 55, and 62°C. Samples were taken at 0, 30, 60, 90, 120, and 150 seconds after reaching the target temperature. A sterile 10 mL syringe was used to collect the sample, which is then placed in sterile Falcon tubes previously sterilized by autoclaving. The samples were immediately cooled in an ice-water mixture and prepared for the culturing stage. As in the previous method, the come-up time is important and was measured in this study [20].

2.2.9. Counting Remaining Colonies

According to FDA regulations, pasteurization must achieve at least a 5-log reduction in pathogenic microorganisms capable of growing in the product [21]. To measure the number of viable *Escherichia coli* bacteria remaining in the carrot juice sample after the applied processes, 1 mL of the sample was serially diluted using nine series of dilution tubes containing 9 mL peptone water or physiological serum diluent. Each dilution from $10^{(-1)}$ to $10^{(-9)}$ was cultivated in triplicate using the pour plate

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method in Plate Count Agar medium. After incubation, the colonies were counted from the dilutions $10^{(-1)}$ to $10^{(-9)}$ [16]. The pour plate method was used to count the microorganisms in the sample, and the plates were incubated upside down for 48 hours to allow colony growth. The colonies were counted using a colony counter, and the number of colonies in each dilution was calculated and reported using the formula in eq. (2) for CFU/mL [15].

Number of colonies (CFU/mL)= number of counted colonies × inverse of dilution factor × inverse of sample volume (Eq. 2)

2.2.10. Statistical Analysis of Data

For statistical analysis, the number of remaining *Escherichia coli* microbes after each treatment was measured, and the logarithm of these data was considered for statistical analysis. All data were examined in three replicates, and if the count difference of a treatment exceeded 15%, the data for that treatment were repeated. The effect of two independent variables, heating method (ohmic and water bath) and heating time (0, 30, 60, 90, 120, 150 seconds), was separately analyzed on the dependent variable of the number of remaining *Escherichia coli* at temperatures of 48, 55, and 62°C using a factorial completely randomized design with SAS software. Mean comparisons were performed using the LSD test at a 5% probability level.

3-Results and Discussion

3.1. Results of Evaluating the Parameter of Remaining *Escherichia coli* After Pasteurization

The research results for the two methods, water bath and ohmic at 100 volts, at three temperatures (48, 55, and 62°C) and six time intervals (0, 30, 60, 90, 120, and 150 seconds) are presented in Table 1. The results of the analysis of variance table indicate that the variables of the number of remaining *Escherichia coli* were significant at the 5% probability level.

Table (1) Analysis of variance of *Escherichia coli* survivors

Sources of changes	Degrees of freedom	average of squares		
		48°C	55°C	62°C
Time	5	0.374 **	0.83 **	25.32 **
Method	1	0.021 **	0.042 *	1.77 **
The interaction of time in the method	5	0.008 **	0.072 **	0.30 **
error	**22**	**0.001**	**0.01**	**0.00**

ns and *: indicate non-significant and significant at the 5% and 1% statistical levels, respectively.

3.2. Results of Ohmic and Water Bath Pasteurization Evaluation

Using a completely randomized design in this study at 100 volts and three temperatures (48, 55, and 62°C) over six times (0, 30, 60, 90, 120, and 150 seconds, with 0 seconds as the control), the results for ohmic and water bath methods are presented in Table 2. The results indicate a significant difference at the 5% probability level. At higher temperatures, the number of remaining bacteria is lower at 55 and 62°C in the water bath method.

Table (2) Mean comparison of *Escherichia coli* survivors in conventional and ohmic methods

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Temperature / Method	48°C	55°C	62°C
Conventional (water bath)	4.80 ± 0.05 [a]	4.51 ± 0.06 [b]	**2.58 ± 0.43 [b]**
Ohmic	4.75 ± 0.06 [b]	4.58 ± 0.10 [a]	**3.03 ± 0.49 [a]**
LSD 5%	0.02	0.06	**0.03**

According to Table 2, it is clear that merely comparing the number of remaining bacteria in the two methods does not indicate the intensity of microbial inactivation and pasteurization. Therefore, two factors, come-up time and heating duration, must be considered. The come-up time, or the time it takes for the treatment to reach the target temperature (48, 55, 62°C) from room temperature (25°C), is important because microbial killing occurs during this period. The longer this time, the more microbes are lost [22].

Table (3) Come up time for conventional and ohmic methods

Temperature / Method	48°C	55°C	62°C
Water bath	1':20"	1':46"	2':18"
Ohmic	0':34"	0':40"	0':52"
Time difference	0':46"	0':56"	1':26"

3.3. Graph of Mean Comparisons of Remaining *Escherichia coli* Over Time

Figures 1, 2, and 3 show the mean of the logarithm of the number of remaining bacteria during the heating process over times 30, 60, 90, 120, and 150 seconds at 48, 55, and 62°C, respectively.

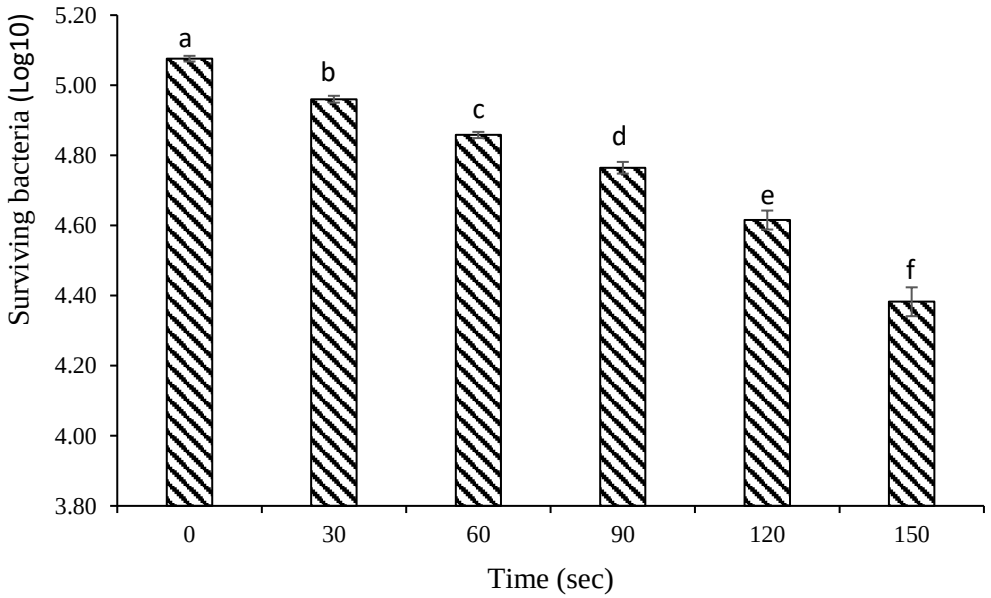

Figure (1) Mean comparison of *Escherichia coli* survivors at different times at 48 °C

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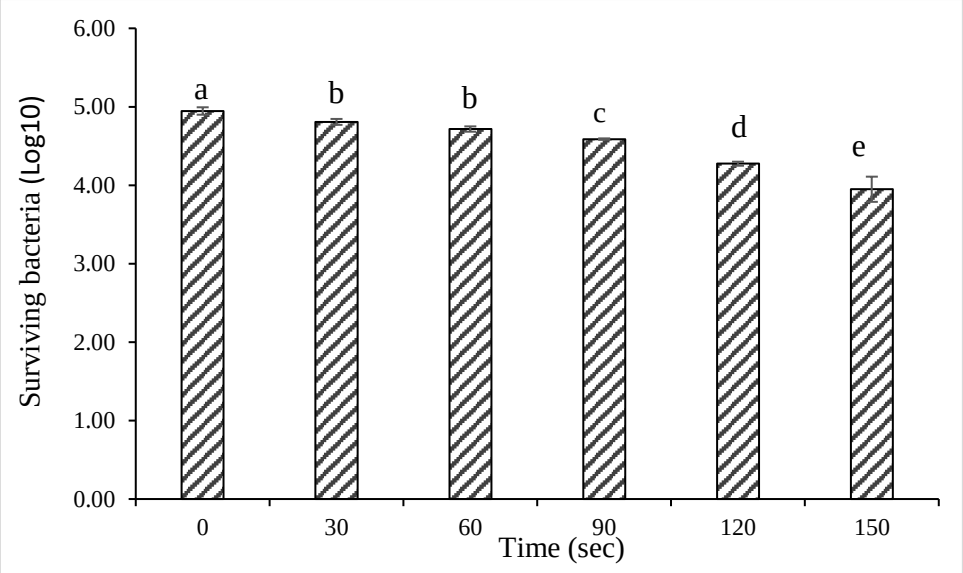

Figure (2) Mean comparison of *Escherichia coli* survivors at different times at 55 °C

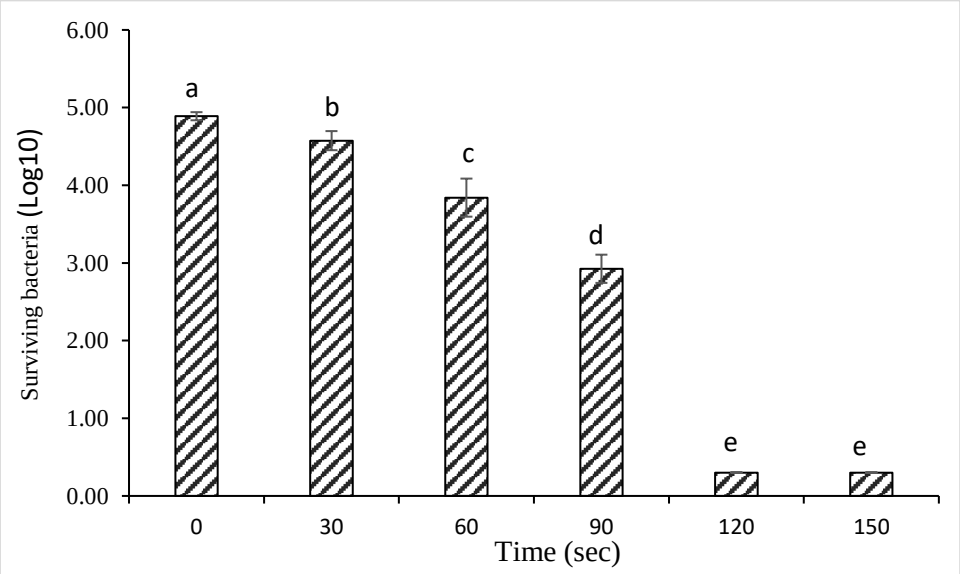

Figure (3) Mean comparison of *Escherichia coli* survivors at different times at 62 °C

According to Figures 1, 2, and 3, the first treatment has a statistically significant difference at the 1% probability level compared to the other treatments. As the temperature increases to 62°C, the number of remaining bacteria decreases such that in the 120 and 150-second treatments at 62°C, no viable bacteria was observed, and the reduction in bacteria was significant at the 1% probability level compared to the other treatments. Overall, the highest microbial inactivation occurred at 62°C and 120 seconds.

3.4. Mean Comparisons of the Interaction of Methods and Time at Different Temperatures with the Effect of Come-up Time

Figures 4 to 6 show the mean comparisons of *Escherichia coli* survivors using ohmic and water bath methods. In each graph, the first

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column represents the control sample at 0 seconds at each temperature, and the other treatments are shown over five times (30, 60, 90, 120, and 150 seconds) for both water bath and ohmic methods at three temperatures (48, 55, and 62°C).

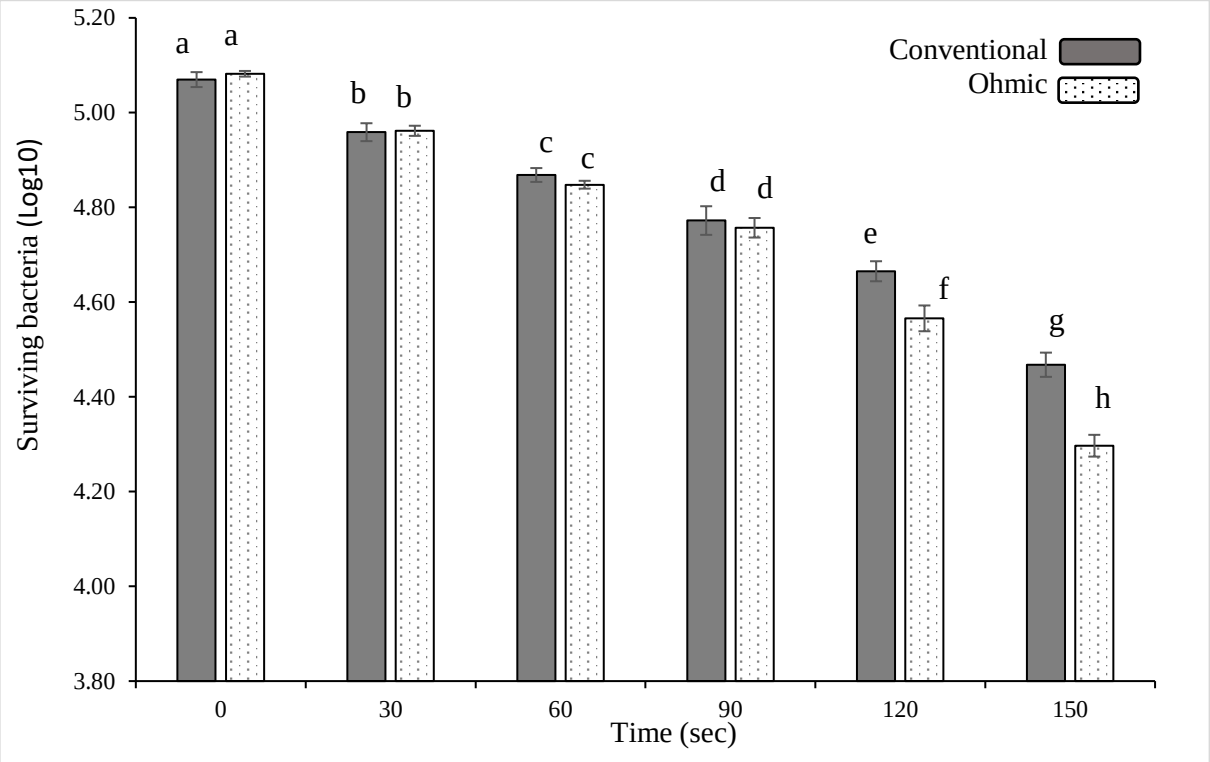

Figure (4) Mean comparison of *Escherichia coli* survivors affected by interaction of time and heating method at 48 °C

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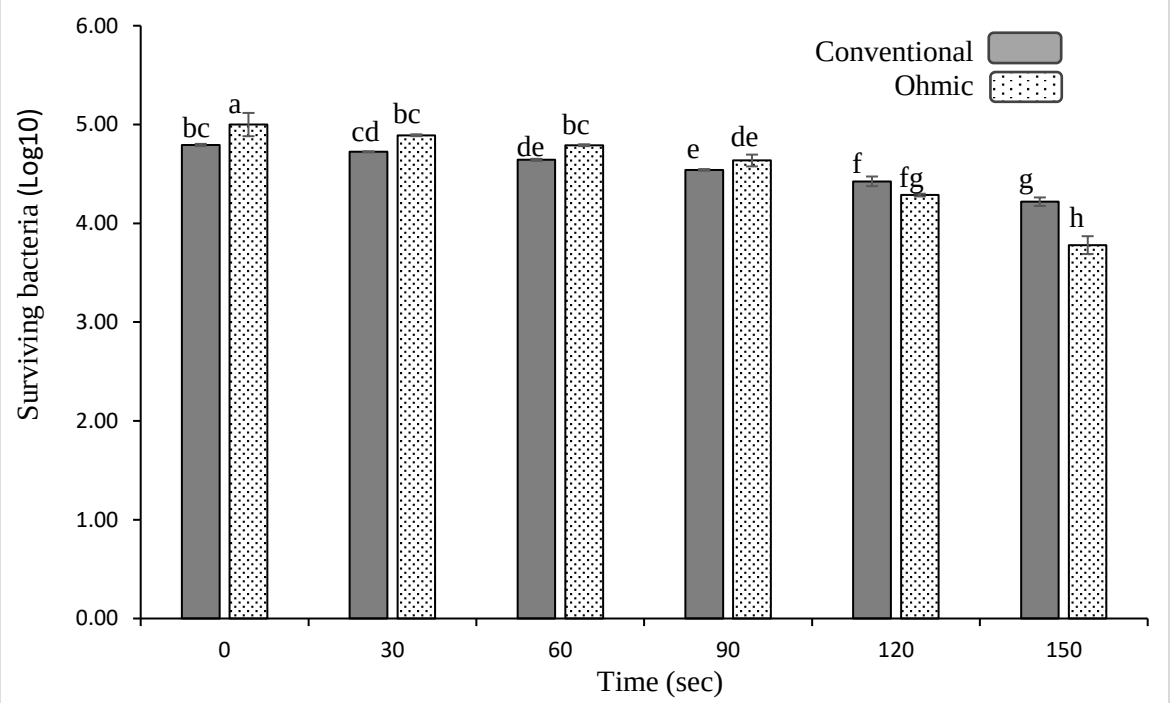

Figure (5) Mean comparison of *Escherichia coli* survivors affected by interaction of time and heating method at 55 °C

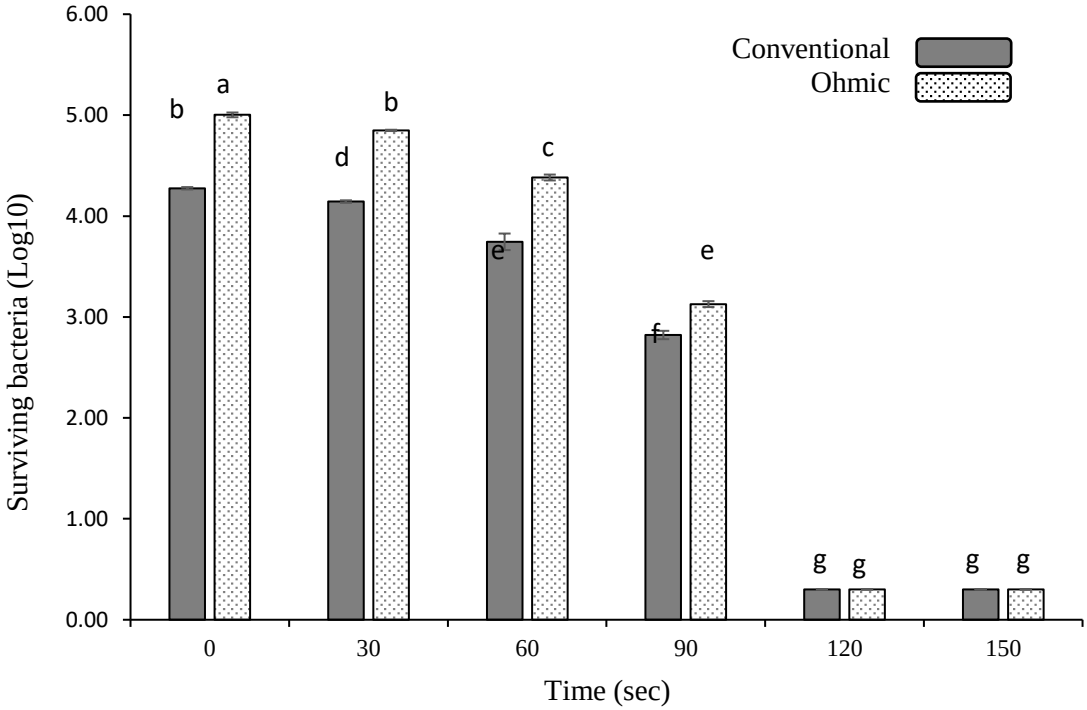

Figure (6) Mean comparison of *Escherichia coli* survivors affected by interaction of time and heating method at 62 °C

Based on the results of the mean comparisons, the first two treatments in each graph are the control samples at that temperature. According to the graphs, there is a significant statistical

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difference between the control samples and the other treatments, indicating a significant reduction in the number of *Escherichia coli* bacteria after thermal treatment.

At 48°C, a significant reduction in the number of surviving microbes is observed over time in both ohmic and traditional methods. In the comparison of the interaction effect of method and time, it is observed that at 0, 30, 60, and 90 seconds, there is no significant statistical difference at the 5% probability level between the water bath and ohmic treatments in each time interval. However, at 120 and 150 seconds, there is a significant statistical difference at the 5% probability level between the ohmic and water bath treatments ($p < 0.05$) (Figure 4).

At 55°C (Figure 5), there is no significant statistical difference between the three treatments: water bath control, 30-second ohmic, and 60-second ohmic, as well as between the three treatments: 60-second water bath, 90-second water bath, and 90-second ohmic. Although no specific statistical difference is observed between the 90-second water bath and ohmic treatments, as well as between the 120-second water bath and ohmic treatments, there is a significant statistical difference at the 5% probability level between the 150-second water bath and ohmic treatments and the other time treatments ($p < 0.05$).

At 62°C, a significant reduction in the number of surviving microbes is observed over time in both ohmic and traditional methods. In the comparison of the interaction effect of method and time, it is observed that at 0, 30, 60, and 90 seconds, there is a significant statistical difference at the 5% probability level between the water bath and ohmic treatments in each time interval ($p < 0.05$), with the count being lower in the traditional method. This is due to the longer come-up time in this method (Table 3), which itself has a lethal effect. At 120 and 150 seconds, there is no significant statistical difference at the 5% probability level between the ohmic and water bath treatments (Figure 6).

There is a significant statistical difference at the 5% probability level ($p < 0.05$) between all ohmic and water bath treatments and the control and 30, 60, and 90-second times separately. However, no significant statistical difference is observed between the last four treatments, i.e., 120-second and 150-second ohmic and water bath treatments. At this temperature, although no significant difference is observed between the water bath control and 30-second ohmic treatments, as well as between the 60-second water bath and 90-second ohmic treatments, there is a significant statistical difference at the 5% probability level ($p < 0.05$) between the 120-second ohmic and water bath treatments and all previous time samples. These results are consistent with those of Kashani et al. (2020) [23].

4- Conclusion

The aim of this study was to inactivate the vegetative form of *Escherichia coli* in carrot juice. Considering the necessity of a 5-log reduction of pathogenic microorganisms capable of growing in the product, the conditions of 62°C for 120 seconds was sufficient to meet the minimum pasteurization requirement in both ohmic and water bath methods. Comparing the come-up time before pasteurization in both ohmic and hot water bath methods, it is important to note that in all cases, the come-up time in the water bath method was longer than in the ohmic method, during which heating and lethal effects occur. Ohmic pasteurization, due to its much shorter time to reach the desired process temperature and complete pasteurization, not only saves time, which in industry indicates higher production in a given time frame and reduced costs, but also indicates energy savings. In most industrial cases, fossil fuels are used to produce steam and generate heat in boilers, but unlike this, ohmic pasteurization is environmentally friendly,

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consuming less energy and time, preserving nutrients, and improving product quality.

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مطالعه اثر حرارت‌دهی اهمیک و حمام آب بر بقای *اشرشیاکلی* در آب هویج

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چکیده

هویج پس از آب‌گیری دارای عمر نگه‌داری بسیار پایینی می‌باشد. پاستوریزاسیون راهی مناسب برای حفظ و تجاری‌سازی این محصول می‌باشد. در این مطالعه از دستگاه اهمیک به عنوان منبع گرمایش استفاده گردید و باکتری اشرشیاکلی به عنوان شاخص کفایت غیرفعال سازی فرم رویشی باکتری برای پاستوریزاسیون انتخاب گردید. آب‌هویج تازه تحت ۳ سطح دمای ٤۸، ۵۵ و ٦۲ درجه سانتی‌گراد و ٦ سطح زمان ۰، ۳۰، ٦۰، ۹۰، ۱۲۰و ۱۵۰ ثانیه با ولتاژ ثابت ۱۰۰ ولت تحت فرآیند حرارتی قرار گرفت و تعداد باکتری‌های بازمانده مورد بررسی قرار گرفتند. همچنین پاستوریزاسیون به روش متعارف حمام آب داغ نیز انجام گرفت و با روش اهمیک مورد مقایسه قرار گرفت که نتیجه‌ی آن کارایی بیشتر روش اهمیک را نشان داد. پاستوریزاسیون به روش اهمیک علاوه بر جلوگیری از اتلاف وقت و انرژی با سرعت بالا باعث غیرفعال شدن فرم رویشی باکتری‌ها در مدت زمان بسیار کمی شده است. تغییرات بازمانده‌های باکتری‌ها با افزایش دما و زمان به وسیله‌ی طرح کاملا تصادفی درسطح احتمال ۱٪ معنی‌دار شد. اثر روش و زمان و اثر متقابل روش و زمان بر کاهش تعداد باکتری‌های زنده‌مانده معنی‌دار بود($P<۰.۰۵$). در دمای ٦۲ درجه سانتی‌گراد بهترین نتیجه برای حرارت‌دهی اهمیک حاصل شد.

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