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# Effects of Tea Polyphenols Combined with Thermosonication on the Population of *Salmonella enterica* in Fresh-Cut Wax Gourd during Storage and Its ANFIS Survival Model

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**Abstract:** Fresh-cut vegetables are exposed to the risk of *Salmonella* spp. contamination. Effective sterilization methods and early warning systems play important roles in ensuring food safety of fresh-cut products. The aim of this study was to evaluate the effect of tea polyphenols (TP) combined with thermosonication (TS) treatment on inactivation of *Salmonella enterica* in fresh-cut wax gourd and to develop and estimate models using adaptive neuro-fuzzy inference system (ANFIS) with different membership functions (MFs) for predicting *S. enterica* population during storage at 25, 10, and 4 °C, respectively. The results showed that both TP and TS treatment can effectively reduce the population of *S. enterica* in fresh-cut wax gourd. The combination of TP (1.0%) and TS (50 °C, 1 min) treatment followed by storage at 4 °C may be a suitable bacteriostatic scheme for the preservation of fresh-cut wax gourd. Fluorescence microscopy analyses indicated that TP and TS treatment could lead to the destruction of the cell membrane, followed by the leakage of cytoplasm, and, finally, cell death. ANFIS with the gaussmf function performed well in modeling and predicting the population of *Salmonella* in fresh-cut wax gourd and provided a powerful tool for modelling and predicting microbe population and the shelf life of food products.

**Keywords:** *Salmonella*; wax gourd; ANFIS; thermosonication; tea polyphenols



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## 1. Introduction

*Benincasa hispida* (Thunb.) Cogn., commonly called wax gourd or winter melon, is a popular vegetable crop widely cultivated in China. It is commonly used in Chinese cuisine, especially in soups, stews, and the chafing dish, as well as in desserts and drinks. Wax gourd is typically oblong or round in shape and can range in size from a few kilograms to over 20 kg. Wax gourd was generally cut into pieces for sizing and weighing when sold in local vegetable markets and supermarkets of China. Recently, fresh-cut or ready-to-cook wax gourd slices became popular due to their convenience for distribution, display, and cooking. However, the fresh-cut process may bring greater risks of microbial contamination compared to thermal processing [1]. *Salmonella enterica*, a common foodborne pathogen, causes gastroenteritis, septicaemia, and even death in humans [2]. It caused approximately 40–80% of foodborne bacterial outbreaks in China, according to different data sources [3,4]. There are no reliable methods widely accepted for inactivating bacteria, extending shelf-life, and compromising vegetable quality [5]. It is, therefore, imperative to develop more preservation methods and to find a reliable prediction model for microbial population in fresh-cut wax gourd.

Tea polyphenols (TP) have antioxidant, anticancer, antiviral, antibacterial, and other physiological functions [6–10]. The antimicrobial activity of TP has been well established, and it has been used in the processing of various foods such as bread [11], cake [12], meat, fish [13–16], and fruit products [17,18] in recent years. Ultrasound has been used for the pasteurization of liquid foods. This is because ultrasonic treatment generates alternating

low- and high-pressure waves in liquids, leading to the formation and violent collapse of small bubbles. This phenomenon is known as cavitation, and it causes strong hydrodynamic shear-forces, intense pressures, and high temperatures during bubble formation and collapse. The use of ultrasound processing combined with sublethal or lethal temperatures, the so called thermosonication (TS), has been shown to have potential applications in food preservation. It leads to better quality (flavor, texture, and appearance) than conventional heat treatment [19–21]. Furthermore, when used with chemical sanitizer, ultrasonic or thermosonic treatment can improve the penetration of the chemical sanitizer through the cellular membrane, resulting in an increased sanitizing efficiency [22–24].

The adaptive neuro-fuzzy inference system (ANFIS), also known as the adaptive-network-based fuzzy inference system, was first proposed [25] as a synergic hybrid system that combines the advantages of artificial neural networks and fuzzy inference systems. It uses a hybrid learning procedure to develop a set of fuzzy if–then rules with appropriate membership functions (MFs) from the input–output data pairs. ANFIS has simple and effective learning algorithms with high training speeds, faster convergence, and better results compared to other learning techniques. Therefore, ANFIS has been used as a modelling and prediction tool in agriculture and food technology [26–28].

The objective of this study was to evaluate the effect of tea polyphenols combined with thermosonication treatment on the survival of *S. enterica* in fresh-cut wax gourd during storage. Additionally, the study also aimed to develop and evaluate an ANFIS model for predicting the population of *S. enterica* using various types of membership functions.

## 2. Materials and Methods

### 2.1. Reagents and Strains

Whole wax gourds were purchased from a local supermarket. Tea polyphenols were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Tryptic Soy Broth (TSB), Buffered Peptone Water (BPW), and Xylose-lysine-tergitol 4 (XLT4) were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). All other reagents were of analytical grade. *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) and *S. enterica* serovar Enteritidis (*S. Enteritidis*) are the most common serotypes associated with human illness in the United States, European countries, and even around the world [29–31]. *S. enterica* serovar Anatum (*S. Anatum*) is also one of the top serotypes most commonly associated with clinical illnesses in humans [32]. Standard strains including ATCC 9270 (*Salmonella enterica* subsp. *enterica*, serovar Anatum), ATCC 13,076 (*Salmonella enterica* subsp. *enterica*, serovar Enteritidis), and ATCC 14,028 (*Salmonella enterica* subsp. *Enterica*, serovar Typhimurium) were deposited in our laboratory [33].

### 2.2. Strain Growth Conditions

Three strains of *Salmonella* spp., ATCC 9270, ATCC 13,076, and ATCC 14,028, were separately cultured in TSB at 37 °C until the exponential phase and then harvested via centrifugation. The bacterial cells were suspended in BPW and serially diluted with BPW. Next, 0.1 mL of each aliquant was spread on an XLT4 agar plate and incubated at 37 °C to count the colonies of *Salmonella*. Subsequently, the three strains were mixed in equal proportions to form a *Salmonella* cocktail with a final cell concentration of approximately 9.0 log CFU/mL [34].

### 2.3. Sample Inoculation, Treatment and Storage

Wax gourds were aseptically peeled and cut into 10 g pieces of a similar size in a laminar flow cabinet. The pieces were then inoculated with 50 µL of the fresh *Salmonella* cocktail using sterile micropipettes and dried for 30 min in the cabinet to ensure that the bacteria adhered sufficiently to the samples. The procedure was repeated on the other side of the samples [35]. After inoculation, the initial levels of *Salmonella* were approximately 7.0 log CFU/g, which were confirmed using plate count analysis.

The TS treatments were performed using a benchtop ultrasound cleaner operating at 40 kHz and 400 W. The inoculated samples were mixed with deionized water in a 1:3 (*w*/*v*) ratio and treated at 40 °C, 50 °C, or 60 °C for 1, 2, or 3 min. Subsequently, the wax gourd samples were immediately immersed in a water bath with 0.5%, 1.0%, or 1.5% (*w*/*v*) tea polyphenols solution (sample: solution = 1:3) at 15 °C for 5 min. Excess liquid was then naturally drained in a laminar flow cabinet at room temperature for approximately 10 min [23].

To investigate the changes in *Salmonella* population during storage, samples treated with TS (50 °C, 1 min) and 1.0% TP were immediately placed in stomacher bags, sealed, and stored at temperatures of 4 ± 1 °C, 10 ± 1 °C, and 25 ± 1 °C. Control samples with an initial *Salmonella* level of approximately 4.6 log CFU/g were kept unsonicated and stored under the same conditions. The populations of *Salmonella* were determined by plate count analysis during storage.

### 2.4. Determination of Texture

The firmness of wax gourd samples was measured using a digital texture analyzer (model GY-4, HANDPI Instruments Co., Ltd., Yueqing, China) with an 8 mm diameter cylinder probe. The peak force (N) required to penetrate wax gourd samples was referred to as the measure of sample firmness [34,36]. All firmness data presented in this study were the average of six replicates.

### 2.5. Enumeration of Salmonella

To recover bacterial cells, 10 g of sample was added to each stomacher bag along with 90 mL of BPW. The samples were pummeled in the stomacher for 60 s twice. The resulting homogenates were serially diluted in BPW, and 0.1 mL of each dilution was plated onto a range of XLT4 agar, which is a selective medium for *Salmonella* and displays specific black or black-centered colonies. The plates were incubated at 37 °C, and the colonies were counted and recorded as log CFU/g [23,33,36].

### 2.6. Fluorescence Microscopy

Fluorescence microscopy analysis was conducted to evaluate the inactivation effect of the combined treatment with TP (1.5%) and TS (3 min at 60 °C). Homogenates generated by stomacher were diluted and stained with 20 µmol/L Hoechst 33,342 and 15 µmol/L propidium iodide (PI). The staining was visualized by exciting with 350 and 488 nm UV light, respectively. The micrographs were captured using the CCD camera of a Nikon 80i fluorescence microscope (Tokyo, Japan).

### 2.7. ANFIS Model

The adaptive neuro-fuzzy inference system utilizes a neural network to generate and optimize decision rules based on Takagi–Sugeno fuzzy inference system. Among the five layers of ANFIS architecture, layer 1 is the fuzzification layer. Nodes in this layer are adaptive nodes with a membership function. There are eight types of MFs that can be used for fuzzification of input data. The parameters of MFs in layer 1 are referred to as premise parameters. Layers 2 and 3 are the rule and normalization layers, respectively, with fixed nodes. Layer 4 is the defuzzification layer. Nodes in this layer are adaptive nodes with a MF. Parameters of nodes in this layer are referred to as consequent parameters. Layer 5 is the output layer with a single fixed node. A hybrid training method, which combines the least-squares and back-propagation algorithms, was employed to fine-tune the premise and consequent parameters of the ANFIS model in the present study.

The ANFIS toolbox of Matlab 2016a was utilized to build ANFIS models for predicting the *Salmonella* population. Each input/output pair contained two inputs (storage temperature and storage days) and one output (*Salmonella* population). In total, 102 experimental data were randomly separated and used for training (60%), checking (18%), and testing (22%) the model, respectively. The prediction performance of the ANFIS model was esti-

mated using the coefficient of determination ($R^2$), root mean squared error (RMSE), and residual predictive deviation (RPD) of the linear regression line between the predicted and experimental values at the training and prediction phases.

### *2.8. Statistical Analysis*

SPSS Statistics software v17.0 were used for the calculation of the means and standard deviations. A Student's *t* test was determined to compare the differences between data groups with a level of significance at $p < 0.05$.

## 3. Results and Discussion

### *3.1. Effect of TP and TS on Salmonella Populations and Wax Gourd Firmness*

*Salmonella* populations in fresh-cut wax gourd pieces treated with TP and TS were monitored using plate count, and the results are shown in Figure 1. It is evident that both TP and TS treatment can effectively reduce the population of *Salmonella* in fresh-cut wax gourd. In general, TP treatments (TS treatment time = 0) reduced the *Salmonella* population by about 0.3–0.7 log CFU/g, and higher concentrations of TP resulted in greater reductions in most cases. However, as TP concentration increased from 1% to 1.5%, the bacteriostatic effect did not improve proportionately. TS treatments (TP concentration = 0) could reduce *Salmonella* population by about 1.5–4.7 log CFU/g, depending on the temperature and time used. Higher temperature and longer time had a greater effect on the reduction of *Salmonella* population. The combination of TP and TS treatments showed better inactivation efficacy than each treatment independently. In this study, the best effect was observed with the combination of TP (1.5%) and TS (3 min at 60 °C), which reduced *Salmonella* population by about 5.5 log CFU/g.

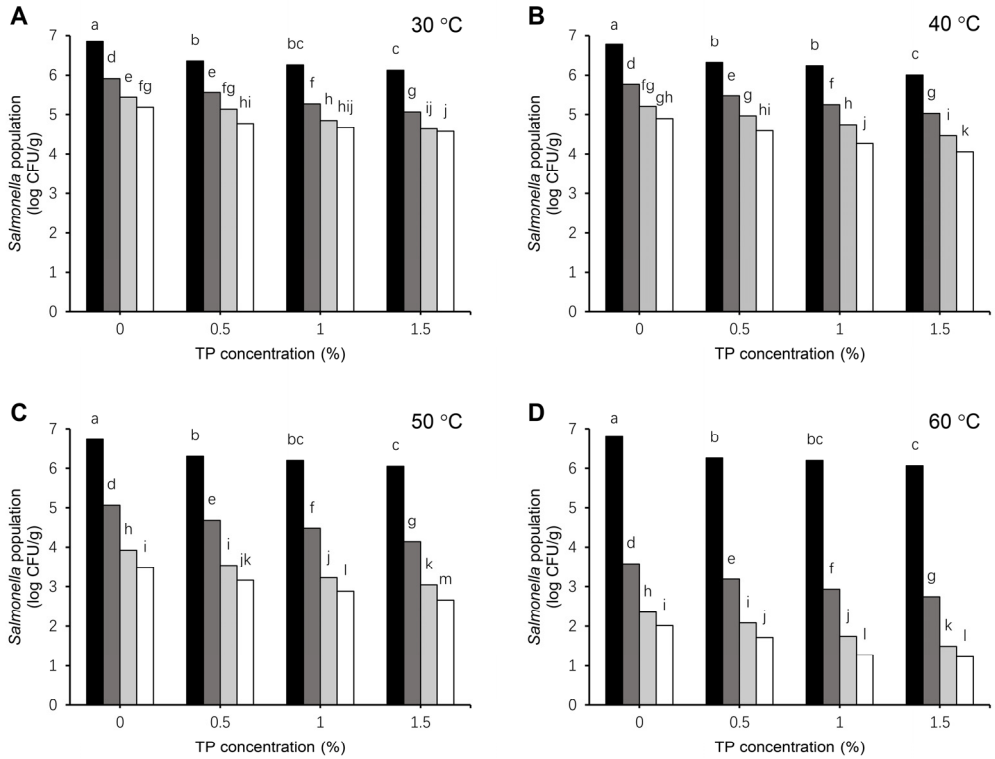


**Figure 1.** Effect of thermosonication treatment with different treatment temperature (30, 40, 50, and 60 °C), treatment time (0, 1, 2, and 3 min), and tea polyphenols concentration (0, 0.5, 1.0, and 1.5%) on *Salmonella* population (log CFU/g). Colors of bars represent treatment time, (Black) 0 min, (Dark grey) 1 min, (Light grey) 2 min, and (White) 3 min. Panels represent *Salmonella* population treated with (**A**) 30 °C, (**B**) 40 °C, (**C**) 50 °C, and (**D**) 60 °C. In each panel, different letters on bars of various colors indicate significant differences at $p < 0.05$ using Student's *t* test.

The firmness of the wax gourd samples was evaluated using a digital texture analyzer, and the results were shown in (Supplementary Materials Figure S1). The results indicated that the firmness of wax gourd was primarily affected by the storage temperature and had little correlation with the TP and TS treatments. The higher the storage temperature, the faster the hardness decreased, particularly when stored at 25 °C, where the firmness of the wax gourd rapidly decreased. After six days of storage, the wax gourd became unmeasurable due to severe spoiling and softening. Further research is needed to fully understand the impact of TP and TS on the quality and sensory properties of wax gourd.

TS has been widely used for pasteurization and *Salmonella* inactivation in various food products. An earlier study evaluating the effect of TS on the survival of *Salmonella* enterica in intact eggs found that the killing effect of TS was temperature dependent, with higher temperatures having a higher killing effect [37]. Another study found that TS at 60 °C had a higher inactivation effect than at 50 °C, and 5 min TS treatment at 60 °C could kill all *Salmonella Enteritidis* cells in mango juice [38]. It was reported that 5 min TS at 60 °C could reduce *Salmonella enterica* serovar Typhimurium on fresh-cut bell peppers by 2–3 log CFU/g [34]. Consistent with previous findings, our results suggested that TS Treatment at 60 °C had a higher inactivation efficacy against *Salmonella enterica*. Furthermore, a 3 min TS treatment at 60 °C could reduce *Salmonella enterica* in fresh-cut wax gourd by about 4.5 log CFU/g, which is better than that in bell pepper but less effective than in mango juice. These differences in the effectiveness of TS treatment may be due to variations in food material matrix, such as state (solid or liquid), pH, organic acids, osmotic pressure, etc.

TP has been used for the preservation of fresh-cut fruit and vegetables, as well as meat and fish products. Combining green tea extracts with atmospheric radio-frequency plasma has been shown to enhance protection against pathogens on fresh-cut dragon fruit [18]. An edible coating made of fish gelatin and TP has been developed and was found to be more effective in preventing spoilage of fish fillets than either treatment alone [39]. To our knowledge, this is the first report on the combined inactivation effect of TP and TS on *S. enterica* in fresh-cut Wax Gourd. The best result was achieved with a combination of 1.5% TP and 3 min TS at 60 °C for *S. enterica* inactivation. Considering that higher temperatures and concentrations of TP may result in increased energy and material costs under real marketing conditions, the combination of TP (1.0%) and TS (50 °C, 1 min) was selected for the wax gourd storage experiment and ANFIS modeling.

Fluorescence microscopy analyses were performed using Hoechst 33,342 and PI staining to evaluate the inactivation effect of the combination of TP (1.5%) and TS (60 °C, 3 min). Hoechst 33,342 is a cell-permeable DNA dye that can stain both living and dead cells, emitting blue fluorescence upon excitation by ultraviolet light. PI, on the other hand, is a nucleic acid dye that cannot penetrate living cells with an intact cell membrane. Upon excitation by ultraviolet light, it emits red fluorescence. As shown in Figure 2, the untreated bacterial cells were only stained with Hoechst 33,342 (blue). However, after the combined treatment with TP and TS, most bacterial cells were stained with both Hoechst 33,342 (blue) and PI (red), and their morphology became blurred. This indicates that the combination of TP and TS can destroy the cell membrane, allowing PI to penetrate the membrane and stain the nucleus. The leakage of cytoplasm, ultimately, leads to cell death. These findings are consistent with previous studies, which have suggested that ultrasound or TS can disrupt cells and enhance sanitization efficiency by facilitating the penetration of chemical sanitizers through the cellular membrane [40–42].

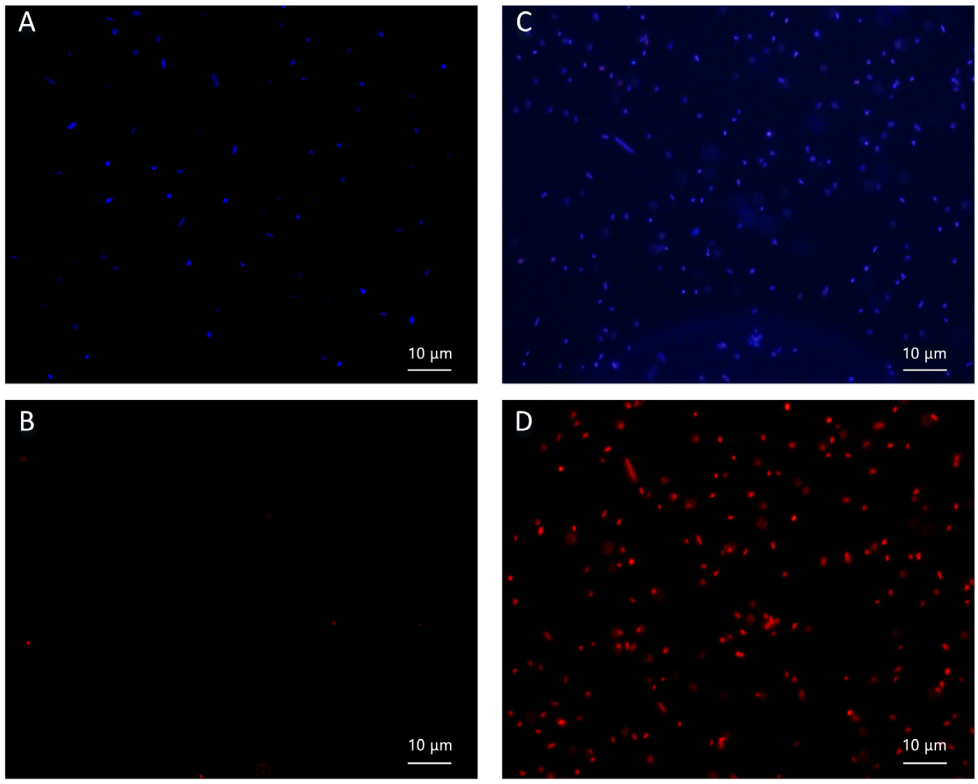


**Figure 2.** Fluorescence microscopy images showing *Salmonella* status of treated samples (1.5% TP combined with 3 min TS at 60 °C) and untreated samples. Hoechst 33,342 staining (blue) showed both live and dead bacterial cells, while PI staining (red) showed only dead bacterial cells. (**A**) an untreated sample stained with Hoechst 33,342, and (**B**) an untreated sample stained with PI. (**C**) TP and TS treated sample stained with Hoechst 33,342, (**D**) and the TP and TS treated sample stained with PI.

### 3.2. *Salmonella* Populations during Storage

*Salmonella*-inoculated wax gourd pieces treated with the combination of TP (1.0%) and TS (50 °C, 1 min) were stored at different temperatures. *Salmonella* populations were monitored using plate count and shown in Figure 3.

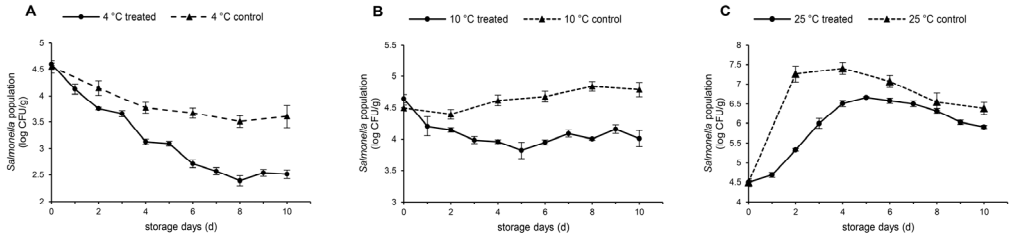


**Figure 3.** *Salmonella* populations (log CFU/g) in fresh-cut wax gourd during storage at different temperatures: 4 °C (**A**), 10 °C (**B**), and 25 °C (**C**), respectively. *Salmonella* populations of treated samples (by TP and TS) were represented as (●) and connected by solid line; *Salmonella* populations of control samples (without treatments) were represented as (▲) and connected by dashed line.

Before treatment, the initial *Salmonella* levels were 6.80 ± 0.04 log CFU/g, confirmed by plate count analysis. After treatment, the *Salmonella* populations were reduced to about 4.58 ± 0.08 log CFU/g. In other words, the combination of TP and TS treatment effectively reduced the population of *Salmonella* in fresh-cut wax gourds by approximately 2.2 log CFU/g. When fresh-cut wax gourd was stored at 25 °C, the *Salmonella* populations of the treated samples significantly increased within the first 4 days, reached a stationary

phase from day 4 to 7, and then declined in the remaining days. The control samples showed similar trends but with a higher maximum of 7.4 log CFU/g on day 4 and an earlier stationary phase on days 2 to 4. When stored at 10 °C, the *Salmonella* populations of the treated samples significantly decreased by about 0.4 log CFU/g within the first 24 h, declined slowly for the first 5 days, and then fluctuated around 4.0 log CFU/g for the rest of the days. The *Salmonella* populations of control samples increased very slowly to a maximum of 4.7 log CFU/g and remained stable. When stored at 4 °C, the populations of *Salmonella* significantly declined within the first 7 days and fluctuated around 2.5 log CFU/g for the remaining days. The *Salmonella* populations of the control samples significantly declined within the first 8 days and then remained stable at 3.5 log CFU/g.

The fate of *Salmonella* varied among different fruits and vegetables. To our knowledge, this is the first report to investigate the fate of *Salmonella* in wax gourd. Strawn and Danyluk 2010 reported the populations of *Salmonella* in mango and papaya [43]. In mango, *Salmonella* populations increased in the first few days and declined till the end of experiment at 25 °C, 12 °C, and 4 °C. In papaya, *Salmonella* populations increased first, remained for days, and declined at 25 °C and 12 °C, while being kept unchanged or declined though the experiment at 4 °C. The results in dragon fruit were similar to papaya [35], although only data in the first 4 days were provided. Sant'Ana et al. reported the growth potential of *Salmonella* in nine ready-to-eat vegetables [44], which kept unchanged or declined in most vegetables when stored at 7 °C, while increasing significantly at 15 °C. In our study, the trends of *Salmonella* populations in the wax gourd of the control samples were similar to those in mango, papaya, and dragon fruit when in storage at 25 °C. However, it increased slowly and reached a stationary phase when stored at 10 °C, which is different from the results in mango and papaya stored at 12 °C. When stored at 4 °C, the fate of *Salmonella* populations in wax gourds was more similar to that in dragon and papaya. The differences in the fate of *Salmonella* population may be due to variations in the nature of the vegetables, including differences in pH, texture, and antimicrobial properties.

*Salmonella* population levels in the treated group were significantly lower than those in the control group during storage at different temperatures. This difference between the groups could be attributed to the combined treatment of TP and TS. TP is a non-volatile bacteriostatic agent derived from tea, which can remain in the vegetable and continuously inhibit *Salmonella* growth. The TS was found to have better bactericidal ability than the use of sonication and thermo treatment alone. When used with a chemical sanitizer such as TP, TS can improve sanitization efficiency. Therefore, the combination of TP and TS demonstrated a good sterilization effect in fresh-cut wax gourd. This rapid assay had the potential to provide a pasteurization method for fresh-cut vegetable processing, further strengthening food safety assurance.

It is widely acknowledged that fresh-cut products require proper refrigeration and strict time and temperature control for preservation. In the food industry and commercial sales, 4 °C and 10 °C are commonly employed for low-temperature storage. Our research findings indicated that low-temperature storage, following TP and TS treatment, could significantly inhibit the growth of *Salmonella* and even reduce the total CFU. The inhibitory effect was more pronounced at 4 °C as compared to 10 °C. However, storing fresh-cut products at room temperature (25 °C) was unsuitable for preservation, as our results confirmed that samples stored at 25 °C experienced texture loss and spoilage within a short period of time even with TP and TS treatment, resulting in the complete loss of commercial value. Furthermore, the oral infection dose of *Salmonella*, which could cause serious intestinal diseases, is known to be at a level of about $10^3$ CFU [45]. However, many countries and regions worldwide have stringent regulations for the absence of *Salmonella* in food to safeguard public health. Therefore, it would be worthwhile to investigate the population changes of *Salmonella* at a low start concentration during the preservation process of wax gourd in future studies.

### 3.3. ANFIS Model

The experimental data of the treatment and control groups were randomly divided into three sets for the training (60%), validation (18%), and testing (22%) the ANFIS model. Each input/output pair consisted of two inputs (storage temperature and storage days) and one output (*Salmonella* population). During the training phase, initial ANFIS models were generated using the grid partition method, and eight different types of input membership functions were tested. The final ANFIS architecture had two membership functions for each input variable and a linear membership function for the output variable, and eight if-then rules were constructed for high-quality prediction. The correlation between experimental and predicted *Salmonella* populations with different membership functions at the training and prediction phases is shown in Figure S2. For ANFIS models with most membership functions, except for trapmf, the data points show a linear distribution in the training and prediction stages, and the regression lines in the training and prediction stages are highly coincident.

The RMSE, RPD, and $R^2$ for the experimental and predicted data during the training and prediction phases are shown in Table 1. It shows that the performance of the ANFIS model was influenced by the type of MFs used. Based on the standard that the RMSE should be minimized and the RPD should be greater than 3, the ANFIS model with the "gaussmf" MF showed better performance during both the training and prediction phases compared to other MFs.

**Table 1.** The root mean squared error (RMSE) and the coefficient of determination ($R^2$) for *Salmonella* populations by ANFIS models generated with different types of input membership functions (MFs) at the training and prediction phases.

| Membership Functions | Training | | | Prediction | | |
|---|---|---|---|---|---|---|
| | RMSE | RPD | $R^2$ | RMSE | RPD | $R^2$ |
| trimf | 0.118636 | 11.8055 | 0.988 | 0.204105 | 6.2084 | 0.985 |
| trapmf | 0.152206 | 9.2017 | 0.9952 | 0.369718 | 3.4274 | 0.9811 |
| gbellmf | 0.132012 | 10.6093 | 0.995 | 0.126194 | 10.0414 | 0.9895 |
| gaussmf | 0.12133 | 11.5433 | 0.9933 | 0.116264 | 10.8990 | 0.9921 |
| gauss2mf | 0.109365 | 12.8062 | 0.9952 | 0.119431 | 10.6100 | 0.9828 |
| pimf | 0.181447 | 7.7188 | 0.9943 | 0.168392 | 7.5251 | 0.9789 |
| dsigmf | 0.186646 | 7.5038 | 0.9957 | 0.141256 | 8.9707 | 0.9861 |
| psigmf | 0.186646 | 7.5038 | 0.9957 | 0.141256 | 8.9707 | 0.9861 |

To visually demonstrate whether the predicted values of each model aligned with the experimental data and general knowledge, the predicted *Salmonella* populations of the ANFIS models with different MFs were depicted as colored surfaces, as shown in Figure S3. It showed that the model with gaussmf produced the smoothest output surface without unnecessary fluctuations, and the curve shape at different temperatures corresponded well with both the experimental data and general knowledge. Therefore, we proposed using the ANFIS model with gaussmf to predict *Salmonella* populations in fresh-cut wax gourd during storage. Both the populations of *Salmonella* observed in the experiments and the populations of *Salmonella* predicted by ANFIS with gaussmf are depicted in Figure 4.

Mathematical models for describing microbe growth, such as the Gompertz model [46], Baranyi model [47], and Huang's model [48], have inherent limitations. Usually, these formulas are proposed to describe usual growth curves rather than constant lines or declined curves. Additionally, the best fitting curves for these formulas are sigmoid-shaped curves, which should consist of the lag, exponential, and stationary phases. Although mathematical models are very suitable for describing growth kinetics and have the advantage of providing the physical or biological meanings of some functions, its application in predicting the complex and variable population curves of microbes may be limited. The ANFIS model is established upon fuzzy inference and learns by an artificial neural

network. It can generate if–then rules automatically, and no relevant background knowledge is required. Therefore, it could be easily applied to various situations, making it a powerful tool for the modelling and prediction of microbe populations and the shelf life of food products. The ANFIS system could play an important role in the early warning of foodborne pathogenic microorganisms.

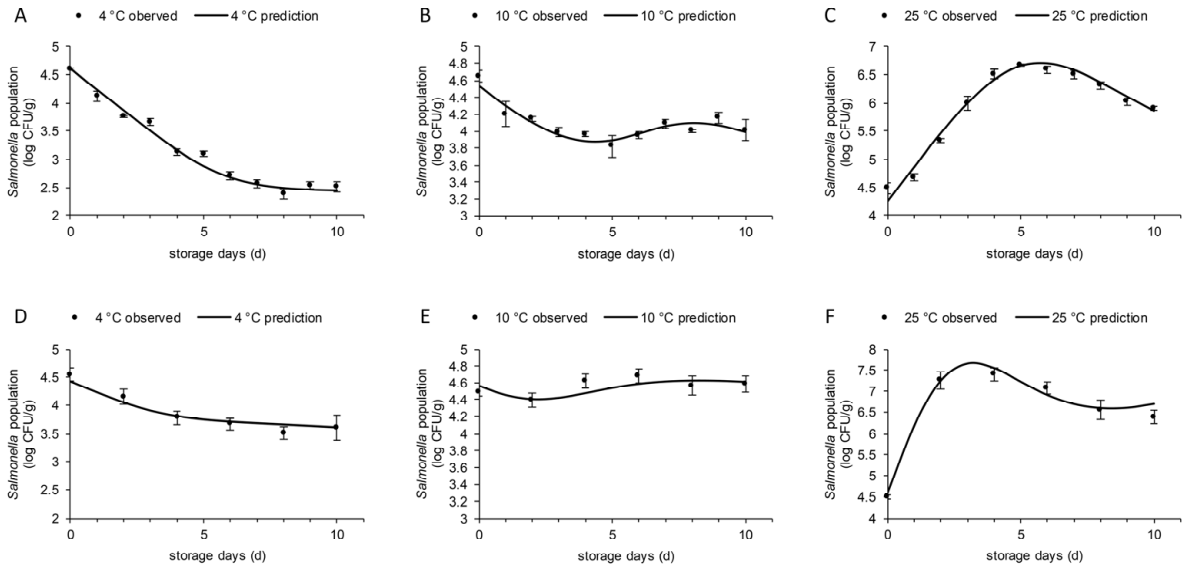


**Figure 4.** The populations of *Salmonella* observed in the experiments (log CFU/g) and the populations of *Salmonella* predicted by ANFIS with gaussmf (log CFU/g) of fresh-cut wax gourd in treated (TP and TS) and control groups during storage at different temperature. (**A**) treated samples stored at 4 °C; (**B**) treated samples stored at 10 °C; (**C**) treated samples stored at 25 °C; (**D**) control samples stored at 4 °C; (**E**) control sample stored at 10 °C; (**F**) control sample stored at 25 °C. *Salmonella* populations observed in the experiments were represented as dots (●); *Salmonella* populations predicted by ANFIS with gaussmf were represented as solid lines.

## 4. Conclusions

Tea polyphenols and thermosonication treatments can effectively reduce the population of *S. enterica* in fresh-cut wax gourds. The higher concentration of TP, the higher the temperature, and the longer time of TS contributed to better inactivation effects. When the concentration of TP is higher than 1%, the benefit of bacteriostatic effect from the increase of TP concentration is significantly reduced. Therefore, taking energy and material costs into account, the combination of TP (1.0%) and TS (50 °C, 1 min) followed by storage at 4 °C may be a suitable bacteriostatic scheme for the preservation of fresh-cut wax gourd. TP and TS treatment could lead to the destruction of cell membrane, then the leakage of cytoplasm, and, finally, cell death. ANFIS with the "gaussmf" function performed well in the modeling and predicting of the population of *Salmonella* in fresh-cut wax gourds. It could be easily applied to various situations and provide a powerful tool for modelling and predicting microbe populations and the shelf life of food products.

Further efforts are needed to deepen our understanding of the underlying mechanism of TP and TS treatment reducing the population of *S. enterica* in fresh-cut wax gourds due to the limitations of our instrumental conditions and experimental experience. This study also prompts the need for further research on the potential application and market value of TP and TS treatment.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13085087/s1, Figure S1: Firmness of fresh cut wax gourd during storage at different temperature: 4 °C (A), 10 °C (B) and 25 °C (C). Firmness of fresh cut wax gourd treated by different conditions were represent with different colors; Figure S2: Correlation between experimental and predicted *Salmonella* population in fresh-cut wax guard treated by thermosonication and tea polyphenols during storage using the ANFIS models with different membership functions at training (*) and prediction (○) phases: (A) 'trimf', (B) 'trapmf', (C) 'gbellmf', (D) 'gaussmf', (E) 'gasuss2mf', (F) 'pimf', (G) 'dsigmf', and (H) 'psigmf'. The coefficients of determination (R2) for experimental populations and prediction data of ANFIS models were shown above (prediction phase) and below (training phase) the regression curve in each panel; Figure S3: Output value (ie, predicted *Salmonella* populations) surface of ANFIS models with different membership functions for TS and TP treated groups. (A) 'trimf', (B) 'trapmf', (C) 'gbellmf', (D) 'gaussmf', (E) 'gasuss2mf', (F) 'pimf', (G) 'dsigmf', and (H) 'psigmf'.

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