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Optimizing the Best Vinegar and Salt Concentration to Reduce Microbial Load in Cauliflower Using Response Surface Methodology

Nafiseh Davati

Assistant Professor, Department of Food Science and Technology, Bu-Ali Sina University, Hamedan, Iran.

n.davati@basu.ac.ir

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Abstract

Today, chemical disinfectants with side effects on health are used to decontaminate vegetables such as cauliflower. These chemical disinfectants can be replaced by natural materials such as salt and vinegar. The purpose of this study was to determine the most convenient way of washing with salt and vinegar solutions to decrease microbial community before usage. The effectiveness of salt (5%, 10.77% and 16.54%) and vinegar (1.5%, 18.25% and 35%) on fungi, *Staphylococcus aureus* and coliform growth using response surface methodology was investigated. In addition, the antibacterial property of disinfectant solutions was determined by agar well diffusion assay. Results showed that washing with a solution containing 35% vinegar and 16.54% salt for five minutes was able to produce a significant 3.81 ± 0.62, 3.38 ± 0.21 and 3.18 Log reduction for fungi, coliform and *S. aureus*, respectively. Evaluation of the antimicrobial potential of vinegar and salt by agar well diffusion assay showed that the combination of salt accompanied with vinegar created a significant inhibitory zone of *Escherichia coli*, *Pseudomonas aeruginosa*, *S. aureus*, and *Bacillus cereus* growth. Using of the solution containing 35% vinegar and 16.54% salt in washing Cauliflower is recommended.

Keywords: Cauliflower, Food Borne Bacteria, Response Surface.

Introduction

Vegetables are one of the major components of the daily diet in Iran and vegetable salad (cauliflower, cucumber, cabbages and lettuce) is one of the daily diets in Iran. Cauliflower has a rough texture, making it an easier area to attach food pathogens. Cauliflower is also in direct contact with soil and irrigating polluted water. Polluted water used for cauliflower raising causes high levels of pollution with faecal coliforms. Cauliflower is cultivated in open lands that are inhabited by animals when compared to tomato, cucumber and other vegetable cultivation lands (Ismail. 2016). Also, vegetables are exposed to post-supply contamination and may become contaminated with different sources such as sewage, soil, air, faece, animal manure, water, animals, harvesting transportation, vehicles and sellers. According to Harris et al. (2003), human and animal enteric pathogens are transferred to vegetables through feces, sewage and contaminated irrigation water. Therefore, the greatest risk of contamination is for vegetables that are consumed raw, especially if the vegetable is grown in soil such as cauliflower. Several food outbreaks are associated with contaminated vegetables such as cauliflower and lettuce (Atter et al. 2014).

Today, to prevent food poisoning, chemical disinfectants are used to decontaminate the surface of fruits and vegetables. Some disinfectants have side effects on health. Cancer disease could be a result of overusing these chemicals such as vegetable disinfectants. Chemical disinfectants can be replaced by natural materials with antibacterial properties such as salt and vinegar to disinfect vegetables with fewer side effects on health. This study aimed to evaluate the hygienic quality of cauliflower obtained from some selected vegetable stores in Hamadan and also determination of the most appropriate washing method with salt and vinegar solutions to decrease the microbial community before consumption.

Materials and methods

Sampling and Sample Supply

Five samples were collected from 5 vegetable stores in Hamadan city in Iran. These were transported to the laboratory using a sterile container. In this study, different pieces of fresh cauliflower were aseptically cut (2 × 2 cm) and analyzed before and after washing in the various disinfectant solutions for five minutes including salt (5%, 10.77% and 16.54%) and vinegar (1.5%, 18.25% and 35%) in triplicate. All solutions were autoclaved at 121°C (15 psi) for 15 minutes.

Central Composite Design

A central composite design involving two independent variables (vinegar and NaCl) and three levels was used to determine the response patterns. The design expert 7.0.0 software was used to create experimental designs, estimate the dependent variable responses, and create the contour and response surface plots. The full design included 13 experiments with 5 central replicates and $\alpha = 1$ (Table 1). The mean log reduction CFU/g for *Staphylococcus aureus*, Coliform and fungi was used as the dependent variable. Experimental data were fitted to a quadratic, linear and quadratic model for the log reduction CFU/g of *S. aureus*, Coliform bacteria, and fungi respectively, and the regression coefficients were determined. The CCD was determined in an optimal vicinity to locate the true optimum concentration of NaCl (X_1) and vinegar (X_2) for antimicrobial activity. The generalized response surface models for four responses were given below as Equations 1 & 2.

Table 1. Central composite experiment design with the two variables and the log reduction in Coliform, *S. aureus* and Fungal counts (CFU/g).

Run	NaCl%	Vinegar%	Log Reduction of *S. aureus* Counts* (CFU/g)	Log Reduction of Coliform Counts* (CFU/g)	Log Reduction of Fungal Counts* (CFU/g)
1	5.00	1.50	0.46 ± 0.18	3.25 ± 0.04	3.59 ± 0.21
2	10.77	18.25	3.18 ± 0.49	3.34 ± 0.33	3.72 ± 0.56
3	10.77	18.25	3.15 ± 0.87	3.33 ± 0.42	3.73 ± 0.31
4	16.54	35.00	3.18 ± 0.63	3.38 ± 0.21	3.81 ± 0.62
5	10.77	18.25	3.18 ± 0.24	3.33 ± 0.98	3.72 ± 0.12
6	10.77	18.25	3.16 ± 0.10	3.34 ± 0.64	3.71 ± 0.01
7	5.00	35.00	1.28 ± 0.27	3.34 ± 0.03	3.77 ± 0.84
8	5.00	18.25	1.03 ± 0.18	3.31 ± 0.84	3.63 ± 0.24
9	10.77	18.25	3.17 ± 0.62	3.33 ± 0.01	3.72 ± 0.03
10	16.54	18.25	3.18 ± 0.10	3.36 ± 0.02	3.78 ± 0.06
11	10.77	35.00	3.18 ± 0.22	3.36 ± 0.15	3.80 ± 0.34
12	10.77	1.50	0.70 ± 0.09	3.30 ± 0.41	3.70 ± 0.29
13	16.54	1.50	0.88 ± 0.75	3.32 ± 0.20	3.74 ± 0.43

*Mean values with standard deviations (±SD) from three experiments

$$\text{Linear model: } Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon \qquad \textbf{Equation (1)}$$

Where Y is predicted response; X_1 (NaCl%) and X_2 (Vinegar%) are input variables; B_0 is a constant; B_1 and B_2 are linear coefficients.

$$\text{Quadratic model: } Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \varepsilon \qquad \textbf{Equation (2)}$$

where Y is predicted response, X_1 (NaCl%) and X_2 (Vinegar%) are input variables; B_0 is a constant; B_1 and B_2 are linear coefficients; B_{12} is cross product coefficients; B_{11} and B_{22} are quadratic coefficients. The coefficient of determination (R^2) and adjusted R^2 (R_{Adj}^2) were used to determine the significance of these models. The final models were displayed as three-dimensional (3D) response surface plots and contour plots by varying two variables.

Validation of the Experimental Design

Factor concentrations were chosen based on the predicted optima of the RSM experimental results described above. The solutions were formulated at optimal concentrations of salt and vinegar as determined above. The antimicrobial activity of these solutions

against fungal load, *S. aureus* and Coliform counts were measured.

Microbiological Analysis

S. aureus of cauliflower was determined by the spread plate method using Baird-Parker (Merck Millipore, Germany) with Egg Yolk Tellurite Emulsion (Merck Millipore, Germany) in accordance with NMKL No. 66, 2009 (Anonymous. 2009), incubated at 37oC for 48 h. Fungus was enumerated by the spread plate method using Sabouraud dextrose agar (Merck Millipore, Germany), incubated at 22-25°C for 5-10 days according to the manufacturer's recommendations. Coliforms were determined by the pour plate method using violet red bile agar (Merckmillipore, Germany), incubated at 30 °C for 24h according to the manufacturer's recommendations.

Test–Bacteria

The antibacterial activity of disinfectant solutions containing salt and vinegar was assessed against four bacterial species including *Escherichia coli* ATCC 25922, *S. aureus* subsp. aureus ATCC 25923, *Bacillus cereus* ATCC 10876 and *Pseudomonas aeruginosa* ATCC 27853. Overnight culture of each bacterial suspension in N.B at 37 °C, was diluted with a sterile physiological solution to 10^8 CFU/g (0.5 McFarland standard) (Valgas et al. 2007; Chen et al. 2019; Moradi et al. 2023).

Screening for Antagonistic Activity

The antibacterial property of disinfectant solutions was screened by agar well diffusion assay (Leroy and De Vuyst, 2004). MH agar (Merck Millipore, Germany) was used for the indicator microorganisms (*E. coli, S. aureus* subsp. aureus, *P. aeruginosa* and *B. cereus*). Petri dishes were mixed with 15 ml of molten agar (1% agar) and 30 µl of the inoculum culture of the indicator microorganism, then wells with a diameter of 5 mm were formed in it. Afterward, 30 µl of each treatment solution was placed in each well. After incubation of the plates for 24 hours at 37 °C aerobically, the growth of microorganisms was examined in terms of inhibitory areas. Inhibition was scored as negative if no zone was observed around the agar well. Each antimicrobial property was associated with the indicated zone of inhibition area (1 mm) (Mathur and Singh, 2005; Bhargava et al. 2015; Davati and Mirzaei, 2020).

A code was allocated to each disinfectant solution containing different concentrations of salt and vinegar as follows:

Code a: 16.54% salt, 1.5% vinegar; **Code b:** 10.77% salt, 35% vinegar; **Code c:** 16.54% salt, 35% vinegar; **Code d:** 5.00% salt, 1.5% vinegar; **Code e:** 16.54% salt, 18.25% vinegar; **Code f:** 10.77% salt, 1.5% vinegar; **Code g:** 10.77% salt, 18.25% vinegar.

Statistical Analysis

All experiments of this study were carried out in triplicates and values were expressed as means with standard deviations. The data of RSM were analyzed using Design-Expert 7.0.0. Figure 1 was drawn using Design-Expert 7.0.0.

Results and Discussion

Evaluation of microbial quality of Cauliflower

In this study, the presence of *S. aureus*, Coliform and fungi was confirmed in Cauliflower. In previous studies, similar organisms were identified from fruits or vegetables (Nwachukwu et al. 2008;

Nwachukwu and Chukwu, 2013; Eni et al. 2010; Rajvanshi. 2010).

The presence of these microorganisms in vegetables creates a public health concern. Fungi commonly cause spoilage of fresh vegetables such as cauliflower and lettuce. These microorganisms could contaminate vegetables through the use of animal manure on the farm, transport vehicles, from other vegetables, spores from the atmosphere, water use in washing the vegetables or from the use of dirty trays and knives for vegetable processing (Beuchat. 1996; De Roever. 1998; Nwachukwu and Chukwu, 2013; Atidégla et al. 2016; Mishra et al. 2017). Ismail (2016), regarding the prevalence of parasitic contamination in salad vegetables, including lettuce, tomatoes, parsley and cucumber collected from stores, proved that the parasitic contamination of salad vegetables was due to irrigation with stool-contaminated water (Ismail. 2016).

Therefore, Cauliflower contamination by pathogenic bacteria is inevitable. *S. aureus* is a type of bacteria commonly found on the skin and in the noses of even some healthy individuals and it is responsible for most food-borne diseases. Human manures are the main source of pathogenic bacteria such as *Salmonella*, *Shigella*, *E. coli*, *Staphylococcus*, *Streptococcus*, and coliforms (Tambekar and Mundhada, 2006; Nwachukwu and Chukwu, 2013; Gundappa and Gaddad, 2016; García and Heredia, 2017; Erhirhie et al. 2020; Balali et al. 2020).

Optimization of solution components by response surface methodology

The CCD was employed not only to study the interactions between the two variables (NaCl, Vinegar) but also to determine their optimal levels. The experimental data listed in Table 1 are analyzed through multiple regressions. The highest antimicrobial activity of these solutions to the highest log reduction of Coliform and fungi observed was 3.38 ± 0.21 CFU/g and 3.81 ± 0.62 CFU/g at run 4, respectively. The highest antimicrobial activity of these solutions to the highest log reduction of *S. aureus* observed was 3.18 CFU/g at run 2, 4, 5, 10 and 11 (Table 1). To determine the optimal antimicrobial activity of these solutions against *S. aureus*, Coliform and fungi, corresponding to the optimum levels of NaCl and Vinegar, models quadratic (equation 3), Linear (equation 4) and quadratic (equation 5) respectively were proposed to calculate the optimum levels of these variables. The linear and quadratic models for the antimicrobial activity of these solutions are shown in the following equation:

$$Y_1 = -2.43+0.56\ X_1+0.13X_2+3.82X_1X_2-0.02X_{12}-3.39X_{22} \quad \text{(Equation 3)}$$

$$Y_2 = 3.24+4.62X_1+2.08X_2 \quad \text{(Equation 4)}$$

$$Y_3 = 3.46+0.02X_1+3.11X_2-2.84X_1X_2-5.59X_1^2+9.40X_2^2 \quad \text{(Equation 5)}$$

Where Y_1, Y_2 and Y_3 were the log reduction (CFU/g) of *S. aureus*, Coliform and fungal count respectively, X_1 is % NaCl and X_2 is % Vinegar.

The results of ANOVA are shown in tables 2, 3 and 4. These models had a very low P value ($P<0.05$), which implied that the models fitted the experimental data very significantly.

The fitness of the model quadratic in equation 3 was examined by the determination coefficient (R^2= 0.95),

which implied that the sample variation of 95% was related to the variables and only 5% of the total variance could not be explained by the model (Table 2). The adjusted determination coefficient (R^2_{Adj}= 0.9142) confirmed the significance of the model, exhibiting a low experimental error and a well-fit regression equation. The F value (1637.78) and P value (0.0001) of lack-of-fit indicated that the lack-of-fit was significantly relative to the pure error.

The fitness of the model Linear in equation 4 was examined by the determination coefficient (R^2= 0.9368), which implied that the sample variation of more than 93% was related to the variables and only less than 7% of the total variance could not be explained by the model (Table 3).

The adjusted determination coefficient (R^2_{Adj}= 0.9242) proved the significance of the model, displaying a low experimental error and a well-fit regression equation. The F value (3.69) and P value (0.1137) of lack-of-fit showed that the lack-of-fit was not significantly associated with the pure error. The fitness of the model quadratic in equation 5 was determined by the coefficient (R^2= 0.9686), which implied that the sample variation of more than 96% was devoted to the variables and only less than 4% of the total variance could not be explained by the model (Table 4). The significance of the model was validated by the adjusted determination coefficient, which showed a low experimental error and a well-fitting regression equation (R^2_{Adj}= 0.9461). The lack-of-fit was considerably related to the pure error, as shown by the F value (8.37) and P value (0.0337). The significance of the model term was evaluated using the values of the P value (Prob > F), which was crucial for comprehending the pattern of reciprocal interactions between the experimental variables for each model.

Table 2 shows that the independent variables, including salt (X_1) and vinegar (X_2) exerted a significant effect on the antimicrobial activity against *S. aureus*. Also, X_1^2 and X_2^2 (*P<0.001*) exerted a significant effect on the antimicrobial activity against *S. aureus*.

Table 3 shows that the independent variables, including salt (X_1) and vinegar (X_2) exerted a significant effect on the antimicrobial activity against Coliform bacteria.

Table 4 shows that the independent variables, including salt (X_1) and vinegar (X_2) exerted a significant effect on the antimicrobial activity against fungi. Also, X_1X_2 and X_2^2 (*P<0.001*) exerted a significant effect on the antimicrobial activity against fungi.

The regression equation was often graphically represented by the 2D contour plot and 3D response surface curve for each of the four responses (Fig. 1). The contour plots' elliptical or saddle nature revealed the importance of the interactions between the corresponding variables for four responses.

Table 2. Variance analysis and parameter estimation for regression equation 1.

Sources of variation	Sum of square	Degree of freedom	Mean square	F value	P value (Prob>F)
Model	15.87	5	3.17	26.58	0.0002
X_1	3.33	1	3.33	27.89	0.0011
X_2	5.23	1	5.23	43.77	0.0003
X_1X_2	0.55	1	0.55	4.59	0.0695

X_1^2	1.71	1	1.71	14.30	0.0069
X_2^2	2.50	1	2.50	20.93	0.0026
Residual	0.84	7	0.12	-	-
Lack of fit	0.84	3	0.28	1637.78	<0.0001
Pure error	6.800E-004	4	1.700E-004	-	-
Cor total	16.70	12	-	-	-

Note: R^2=0.9500; R_{Adj}^2=0.9142

Table 3. Variance analysis and parameter estimation for regression equation 2.

Sources of variation	Sum of square	Degree of freedom	Mean square	F value	P value (Prob> F)
Model	0.012	2	5.808E-003	74.15	< 0.0001
X_1	4.267E-003	1	4.267E-003	54.47	< 0.0001
X_2	7.350E-003	1	7.350E-003	93.83	< 0.0001
Residual	7.833E-004	10	7.833E-005	-	-
Lack of fit	6.633E-004	6	1.106E-004	3.69	0.1137
Pure error	1.200E-004	4	3.000E-005	-	-
Cor total	0.012	12	-	-	-

Note: R^2=0.9368; R_{Adj}^2=0.9242

Table 4. Variance analysis and parameter estimation for regression equation 3

Sources of variation	Sum of square	Degree of freedom	Mean square	F value	P value (Prob> F)
Model	0.045	5	8.974E-003	43.16	< 0.0001
X_1	0.019	1	0.019	92.66	< 0.0001
X_2	0.020	1	0.020	98.19	< 0.0001
$X_1 X_2$	3.025E-003	1	3.025E-003	14.55	0.0066
X_1^2	9.576E-004	1	9.576E-004	4.61	0.0690
X_2^2	1.922E-003	1	1.922E-003	9.24	0.0188
Residual	1.455E-003	7	2.079E-004	-	-
Lack of fit	1.255E-003	3	4.185E-004	8.37	0.0337
Pure error	2.000E-004	4	5.000E-005	-	-
Cor total	0.046	12	-	-	-

Note: R^2=0.9686; R_{Adj}^2=0.9461

Figure 1 shows the response surface plots and corresponding contour plots created by the predicted models for the antimicrobial properties of disinfectant

solutions. From the response surface plots, it was easy to understand the interactions between the two factors, NaCl and vinegar, and also to determine their optimal values.

The highest antimicrobial activity in salt and vinegar for 3.18 (CFU/g) log reduction of *S. aureus* could be observed at 35% vinegar and 16.54% salt; 18.25% vinegar and 10.77% salt; 18.25 % vinegar and 16.54% salt; 35% vinegar and 10.77% salt (Fig. 1 (A and B)). The highest antimicrobial activity of salt and vinegar for 3.38 (CFU/g) log reduction of Coliform bacteria could be observed at 16.54 % NaCl and 35 % vinegar (Fig. 1 (C and D)).

The maximal antimicrobial activity of salt and vinegar for 3.81 (CFU/g) log reduction of fungal counts could be observed at 16.54 % NaCl and 35 % vinegar (Fig. 1 (E and F)).

The high salt concentration solution causes plasmolysis or shrinkage of plant tissue such as vegetables. Also, a higher concentration of acetic acid (vinegar) causes to change taste and odor of vegetables. Therefore, because of salt and vinegar effects at high concentrations on textural and organoleptic (taste and odor) properties of cauliflower, according to equations (1 and 2), we predicted that the maximum desirable log-reduction of coliform, *S. aureus* and fungi include 3.33, 3.19 and 3.72 CFU/g, respectively could be achieved at low desirable levels 10.67 % NaCl and 20.80 % vinegar. Verification experiments were accomplished by using the optimized conditions and gave log-reduction of coliform, *S. aureus* and fungi including 3.36, 3.15 and 3.65 CFU/g respectively, which were closer to the predicted response. Therefore, these results corroborated the predicted values and the effectiveness of the models. This solution containing 10.67 % salt and 20.80 % vinegar could be a good alternative instead of chemical disinfectants with side effects on health.

Figure 1 (A and B) shows the log reduction of *S. aureus* growth in solution containing a high salt percentage and low vinegar percentage is lower than in solution containing a high vinegar percentage and low salt percentage. Also, table 2 shows that vinegar is more effective than salt (P value= 0.0003) and exerts a significant effect on the antimicrobial activity against *S. aureus*.

In past studies, the tolerance of *S. aureus* to high concentrations of sodium chloride has been shown (Parfentjev and Catelli, 1964). Based on the results, the vinegar on log reduction of fungal load and coliforms is more effective than salt (Fig. 1, C, D, E and F). Hosein and et al (2011) reported a reduction in the intracellular pH with increasing acetic acid percentage (Hosein et al. 2011). Similar results were observed by Atter and et al (2011), who found disinfection of green cabbage with 50% vinegar solution was most effective in decreasing the microbial community on the samples tested followed by 5% salt solution compared to tap water (Atter et al. 2014). Nwachukwu and Chukwu (2013) reported that disinfection of vegetables with vinegar was found to significantly decrease their microbial community compared to sodium benzoate and potassium sorbate (Nwachukwu and Chukwu, 2013).

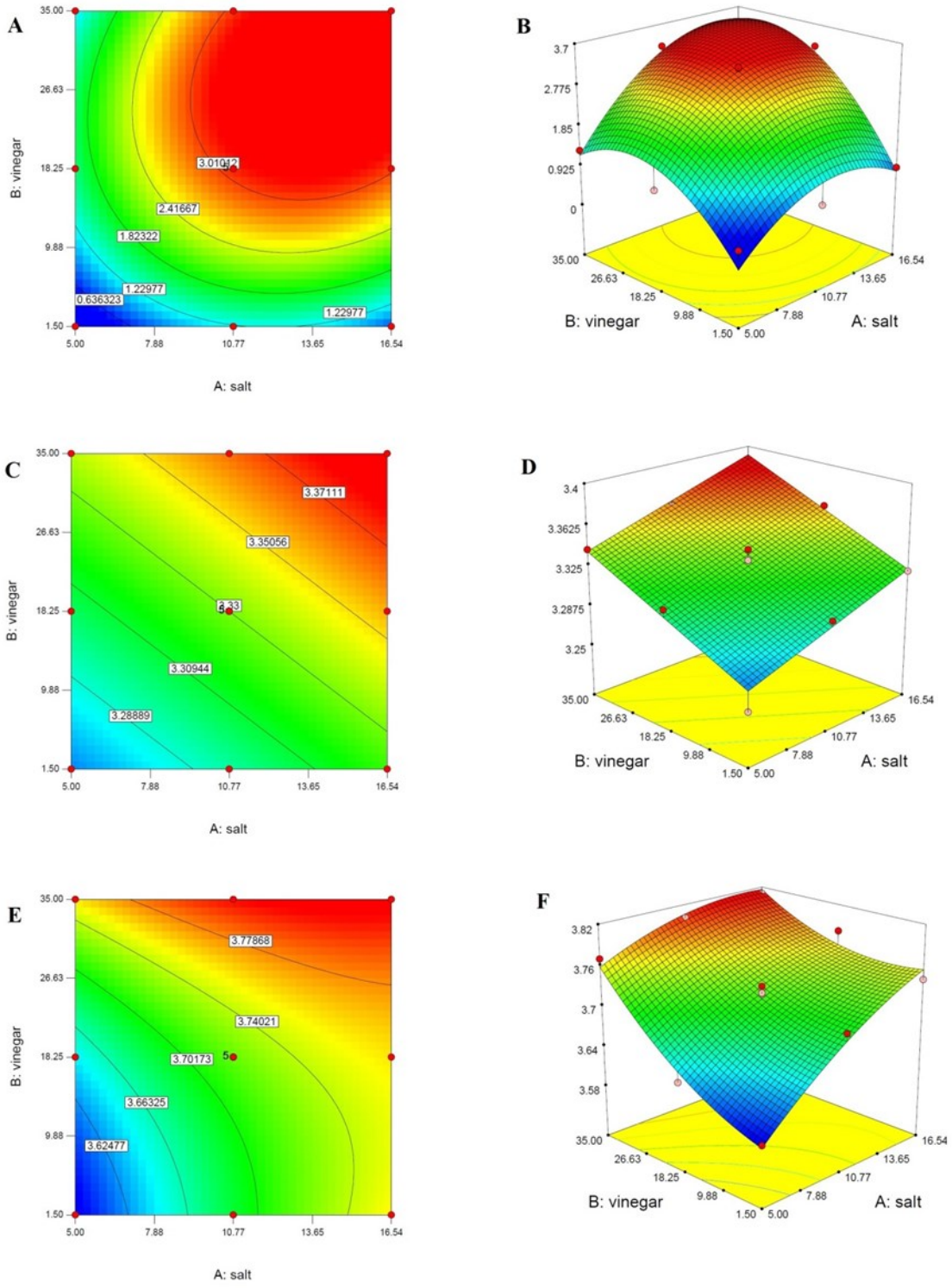

Figure 1. Two-dimensional contour diagram and three-dimensional replication level of interaction for antimicrobial efficacy of salt and vinegar against (A and B) *S. aureus*, (C and D) Coliform and (E and F) Fungi.

Evaluation of antimicrobial effects of salt and vinegar against pathogenic bacteria using well diffusion agar assay

Following the optimization of disinfectant solution components (salt and vinegar), the antimicrobial effects of these solutions using well diffusion agar assay were evaluated to better understand their disinfection power against pathogenic bacteria. After incubation, no bacterial growth was observed in the area around the well containing disinfectant solution. The diameter of the inhibition zone determined the antibacterial activity of this solution. In our experiment, it was found that the diameter of the inhibition zone increases with high concentrations of salt and vinegar. The inhibition zone was not observed in the case of the control well (without any disinfectant solution). The maximum diameter of the inhibition zone was approximately found to be 5 mm with solutions b, e, c and g. According to our experimental results, the disinfectant solution with high concentrations of salt and vinegar indicates that these solutions have effective antibacterial activity against pathogenic bacteria (Fig. 2).

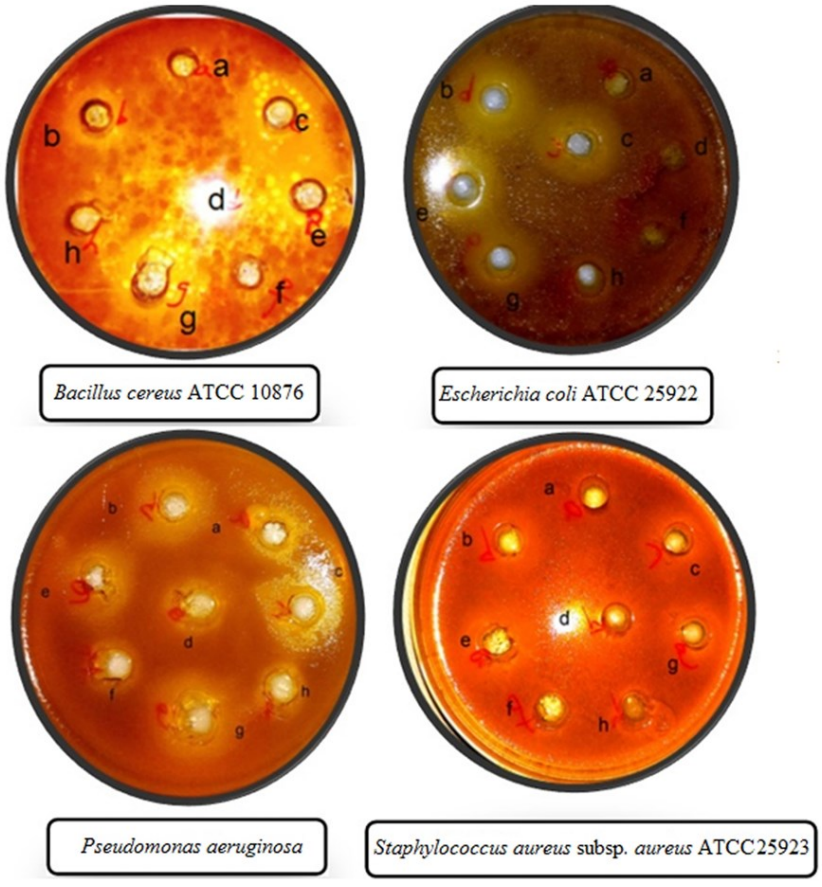

Figure 2. Well diffusion assay showing antimicrobial effect of salt and vinegar on pathogenic bacteria.

Salt removes water from the cells of food and bacteria using the osmosis process. Reducing the amount of water activity limits the growth of bacteria. The antimicrobial activity of vinegar could be attributed to the presence of phenolic compounds and organic acids, especially acetic acid. Several studies indicated the antimicrobial effects of organic acids such as acetic acid against numerous microorganisms (Wali and Abed, 2019; Adamczak et al. 2019; Ousaaid et al. 2021).

Acetic acid according to other organic acids has a lasting impact on preventing the growth of harmful microbes. Organic acids' capacity to release protons H^+ into cells lowers the intracellular pH, which causes the bacterial cell membrane to be destroyed (Zhang et al. 2011). On the other hand, a high concentration of hydrogen ions causes cell macromolecules to protonate, destabilizing microorganisms and ultimately leading to their death (Zhang et al. 2011).

Active transport is used to expel protons, which uses up the energy required for microorganisms to develop normally. (Cherrington et al. 1991).

In this context, phenolic compounds present in vinegar could also be involved in inhibiting the growth of microbes. Polyphenols could disrupt the integrity of cell membranes, altering their permeability (Bouarab-Chibane et al. 2019) and lowering extracellular pH (Pernin et al. 2019). Bioactive compounds interact with various intramolecular components of cells (Botton et al. 1990), creating complexes and influencing the processes of protein synthesis and energy production (Djilani and Dicko, 2012).

Conclusion

This study has shown that Cauliflower because of direct contact with soil and irrigation by wastewater irrigating was contaminated with microorganisms which are responsible for most foodborne diseases. In our study, statistically based experimental designs were confirmed to be powerful tools for optimizing solutions containing salt and vinegar with antimicrobial activity against some pathogenic bacteria to disinfect of vegetables such as cauliflower. Some safe disinfectants such as salt and vinegar should be used to reduce the microbial load of cauliflower. Based on past similar research and our study, vinegar and salt have antimicrobial properties against pathogenic bacteria. The optimized solution of 10.67 % NaCl and 20.80 % vinegar with the least effects on the organoleptic properties of cauliflower could reduce coliform, *S. aureus* and fungi count by about 3.33, 3.19 and 3.72 log CFU/g respectively. The predicted values were in good agreement with the experimental results in the validation experiments, confirming the accuracy of the models. Therefore, the use of this optimized solution containing vinegar and salt in washing cauliflower is recommended. It should be noted that more contact time of solution containing salt and vinegar with cauliflower could also lead to more reduction of bacterial load. Therefore, we propose that the effect of contact time of cauliflower with disinfectant solution should be examined in future research.

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References منابع

1. **Adamczak A., Ożarowski M., Karpiński TM. (2019).** Antibacterial activity of some flavonoids and organic acids widely distributed in plants. J. Clin. Med. 9(1): 109.

2. **Anonymous. 2009.** Nordic Committee on Food Analysis. Coagulase positive staphylococci. Enumeration in foods. Method no 66. 5.Ed.

3. **Atidégla SC., Huat J., Agbossou EK., Saint-Macary H., Glèlè Kakai R. (2016).** Vegetable contamination by the fecal bacteria of poultry manure: case study of gardening sites in Southern Benin. Int. J. Food Sci. PMC4812396

4. **Atter A., Amewowor D., Amoa-Awua W. (2014).** The effectiveness of water, salt and vinegar in reducing the bacteria population in fresh green cabbage. Food. Sci. Qual. Manag. 28: 29-35.

5. **Balali GI., Yar DD., Afua Dela VG., Adjei-Kusi P. (2020).** Microbial contamination, an increasing threat to the consumption of fresh fruits and vegetables in today's world. Int. J. Microbiol. 2020(1).

6. **Beuchat LR. (1996).** Pathogenic microorganisms associated with fresh produce. J. Food. Prot. 59(2): 204-16.

7. **Bhargava K., Conti DS., da Rocha SR., Zhang Y. (2015).** Application of an oregano oil nanoemulsion to the control of foodborne bacteria on fresh Cauliflower. Food Microbiol. 47: 69-73.

8. **Botton B., Breton A., Febre M., Gauthier S., Guy P., Larpent JP., Reymond P., Sanglier JJ., Vayssier Y., Veau P. (1990).** Useful and harmful moulds. Industrial importance. (Ed. 2).

9. **Bouarab-Chibane L., Forquet V., Lantéri P., Clément Y., Léonard-Akkari L., Oulahal N., Degraeve P., Bordes C. (2019).** Antibacterial properties of polyphenols: characterization and QSAR (Quantitative structure–activity relationship) models. Front Microbiol. 10: 829.

10. **Chen CC., Lai CC., Huang HL., Huang WY., Toh HS., Weng TC., Chuang YC., Lu YC., Tang HJ. (2019).** Antimicrobial activity of *Lactobacillus* species against carbapenem-resistant *Enterobacteriaceae*. Fron Microbiol. 10: 789.

11. **Cherrington CA., Hinton M., Mead GC., Chopra I. (1991).** Organic acids: chemistry, antibacterial activity and practical applications. Adv. Microb. Physiol. 32: 87-108.

12. **Davati N. and Ali Mirzaei S. (2020).** Application of Artemisia absinthium essential oil to the control of pathogenic bacteria growth on fresh Cauliflower. FSCT. 17 (98): 85-96.

13. **De Roever C. (1998).** Microbiological safety evaluations and recommendations on fresh produce. Food Control. 9(6): 321-47.

14. **Djilani A. and Dicko A. (2012).** The therapeutic benefits of essential oils. Nutrition, Well-Being and Health. 7: 155-179.

15. **Eni AO., Oluwawemitan IA., Solomon OU. (2010).** Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria. Afr. J. Food Sci. 4(5): 291-6.

16. **Erhirhie EO., Omoirri MA., Chikodiri SC., Ujam TN., Kesiena EE., Oseyomon JO. (2020).** Evaluation of microbial quality of vegetables and fruits in Nigeria: A review. IJNS. 5(3): 99-108.

17. **García S. and Heredia N. (2017).** Microbiological safety of fruit and vegetables in the field, during harvest, and packaging: A global issue. Global Food Security and Wellness. 27-48.

18. **Gundappa M. and Gaddad SM. (2016).** Prevalence of Salmonella, Shigella and E. coli in vegetables of various markets in Kalaburagi (India). IJONS. 6(35): 0976-0997.

19. **Harris L., Farber J., Beuchat L., Parish M., Suslow T., Garrett E. (2003).** Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh cut produce. Compr. Rev. Food Sci. Food Saf. 2(s1): 78-141.

20. **Hosein AM., Breidt F., Smith CE. (2011).** Modeling the effects of sodium chloride, acetic acid, and intracellular pH on survival of *Escherichia coli* O157: H7. Appl. Environ. Microbiol. 77(3): 889-95.

21. **Ismail Y. (2016).** Prevalence of parasitic contamination in salad vegetables collected from supermarkets and street vendors in Amman and Baqa'a–Jordan. Pol. J. Microbiol. 65(2): 201-207.

22. **Leroy F. and De Vuyst L. (2004).** Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci. Technol. 15(2): 67-78.

23. **Mathur S. and Singh R. (2005).** Antibiotic resistance in food lactic acid bacteria-a review. Int. J. Food Microbiol. 105(3): 281-95.

24. **Mishra A., Pang H., Buchanan RL., Schaffner DW., Pradhan AK. (2017).** A system model for understanding the role of animal feces as a route of contamination of leafy greens before harvest. Appl. Environ. Microbiol. 83(2): e02775-16.

25. **Moradi A., Davati N., Emamifar A. (2023).** Effects of *Cuminum cyminum* L. essential oil and its nanoemulsion on oxidative stability and microbial growth in mayonnaise during storage. Food Sci Nutr. 1: 4781–4793.

26. **Nwachukwu E. and Chukwu C. (2013).** Effect of chemical treatments on the microbial load of fruits and vegetables. Int. J. Appl. Microbiol. Biotechnol. Res. 1(3): 16-9.

27. **Nwachukwu E., Ezeama C., Ezeanya B. (2008).** Microbiology of polyethylene-packaged sliced watermelon (*Citrullus lanatus*) sold by street vendors in Nigeria. Afr. J. Microbiol. Res. 2(8): 192-5.

28. **Ousaaid D., Laaroussi H., Bakour M., Ennaji H., Lyoussi B., El Arabi I. (2021).** Antifungal and antibacterial activities of apple vinegar of different cultivars. Int. J. Microbiol.

29. **Parfentjev I. and Catelli AR. (1964).** Tolerance of *Staphylococcus aureus* to sodium chloride. J. Bacteriol. 88(1): 1-3.

30. **Pernin A., Guillier L., Dubois-Brissonnet F. (2019).** Inhibitory activity of phenolic acids against Listeria monocytogenes: deciphering the mechanisms of action using three different models. Food Microbiol. 80: 18-24.

31. **Rajvanshi A. (2010).** Bacterial load on street vended salads in Jaipur city, India. Internet J. Food Saf. 12(1): 136-9.

32. **Tambekar D. and Mundhada R. (2006).** Bacteriological quality of salad vegetables sold in Amravati city (India). J. Biol. Sci. 6(1): 28-30.

33. V**algas C., Souza SMd., Smânia EF., Smânia Jr A. (2007).** Screening methods to determine antibacterial activity of natural products . Braz. J. Microbiol. 38(2): 369-80.

34. **Wali MK., and Abed MM. (2019).** Antibacterial activity of acetic acid against different types of bacteria causes food spoilage. Plant Arch. 19(1): 1827-1831.

35. **Zhang J., Tian ZG., Wang JH., Wang AR. (2011).** Advances in antimicrobial molecular mechanism of organic acids. Acta veterinaria et zootechnica sinica- Chin. J. Anim. Sci. Vet. 42(3): 323-328.

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بهینه‌سازی بهترین غلظت سرکه و نمک برای کاهش بار میکروبی گل کلم با استفاده از روش سطح پاسخ

نفیسه دعوتی

استادیار، گروه علوم و صنایع غذایی، دانشکده صنایع غذایی بهار، دانشگاه بوعلی سینا، همدان، ایران.

n.davati@basu.ac.ir

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صفحه ۱۰۶-۹۳

چکیده

امروزه از ضدعفونی‌کننده‌های شیمیایی با عوارض جانبی جهت رفع آلودگی سبزی‌هایی مانند گل کلم استفاده می‌شود. این ضدعفونی‌کننده‌های شیمیایی را می‌توان با مواد طبیعی مانند نمک و سرکه جایگزین کرد. هدف از این مطالعه تعیین مناسب‌ترین روش شستشو با محلول‌های نمک و سرکه جهت کاهش جامعه میکروبی قبل از مصرف گل کلم بود. اثر نمک (۵٪، ٪ ۱۰/۷۷ و ٪ ۱۶/۵۴) و سرکه (٪ ۱/۵، ٪ ۱۸/۲۵ و ٪ ۳۵) بر رشد قارچ‌ها، *استافیلوکوکوس اورئوس* و کلی‌فرم با استفاده از روش سطح پاسخ بررسی شد. همچنین خاصیت ضد میکروبی محلول‌های ضد عفونی کننده با روش انتشار از چاهک در آگار بررسی شد. نتایج نشان داد که شستشوی گل کلم با محلول حاوی ٪ ۳۵ سرکه و ٪ ۱۶/۵۴ نمک به مدت ۵ دقیقه باعث شد به ترتیب ۰/۶۲ ± ۳/۸۱، ۰/۲۱ ± ۳/۳۸ و ۳/۱۸ کاهش لگاریتم در رشد قارچ ها، کلی‌فرم و *استافیلوکوکوس اورئوس* ایجاد شود. ارزیابی پتانسیل ضد میکروبی سرکه و نمک با استفاده از روش انتشار از چاهک در آگار نشان داد که ترکیب نمک همراه با سرکه باعث مهار قابل توجه رشد در *اشریشیا کلی*، *استافیلوکوکوس اورئوس*، *سودوموناس آئروژینوزا* و *باسیلوس سرئوس* شد. استفاده از محلول حاوی ٪ ۳۵ سرکه و ٪ ۱۶/۵۴ نمک برای شستشوی گل کلم توصیه می‌شود.

واژه‌های کلیدی: گل کلم، باکتری‌های عفونت‌زای غذایی، سطح پاسخ.