Food Science and Engineering
http://ojs.wiserpub.com/index.php/FSE/

Research Article

Optimization of Fruit Juice Preservation Utilizing Chitosan and Chitosan Nanoparticle: A Central Composite Design

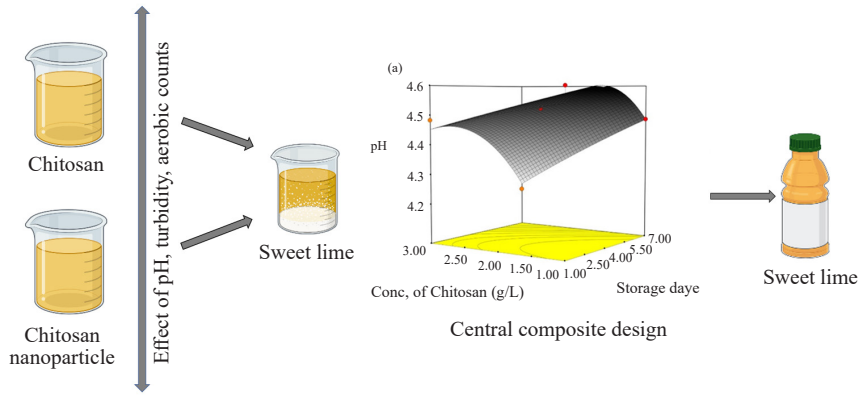
Gurupriyadarsini Annadurai[1], Lakshana Sri Ravichandran Mangayarkarasi[1], Minnalkodi Sivakavinesan[2], Jenson Samraj Jeyaprakash[2*], Mala Madasamy[3], Selvam Jayapandi[4], Annadurai Gurusamy[2]

[1]Department of Food Technology, Center for Biotechnology, Anna University, Chennai, India
[2]Department of Environmental Science, Sri Paramakalyani Centre for Excellence in Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, India
[3]Department of Biotechnology, Faculty of Science and Humanities, Sri Ramaswamy Memorial University, Kattankulathur, Kancheepuram, India
[4]Centre for Nano and Material Sciences, Jain University, Jain Global Campus, Jakkasandra, Ramanagaram, Bangalore, India
E-mail: jj4881@srmist.edu.in

Received: 3 January 2024; **Revised:** 14 March 2024; **Accepted:** 11 April 2024

Abstract: In this study, the shelf life of sweet lime juice was tested using chitosan and chitosan nanoparticles produced through the Ionic-gelation process. The results showed that the chitosan nanoparticles had a greater impact on extending the shelf life than chitosan alone. The pH, turbidity, and aerobic count of the juice were measured after the chitosan and chitosan nanoparticles were added, and response surface methodology was used to optimize these factors. The optimal pH range for chitosan was between 3.0 and 4.6, and for chitosan nanoparticles it was between 3.0 and 4.9. The maximum turbidity occurred at a chitosan concentration of 0.5-2.5 g/L and storage days of 1.0-2.0. The results were found to be significant through analysis of variance, and the model had a high level of significance and good fit according to the determination coefficient.

Graphical abstract



DOI: https://doi.org/10.37256/fse.5120244205

***Keywords*:** fruit juice preservation, chitosan, ionic-gelation, chitosan nanoparticle, response surface methodology, statistical analysis

1. Introduction

Foodborne illnesses are a serious issue, as they cause the deaths of thousands of people each year due to bacteria, viruses, and parasites [1]. The sweet lime (*Citrus limetta*) fruit is a popular food in Asia, known for its high Vitamin C and energy content. However, the increasing number of illnesses related to juice consumption caused by chemical preservatives has led scientists to search for alternative, natural preservation methods. One such alternative is chitosan, a natural biopolymer that has been explored for its potential use in food preservation, antioxidant properties for juices, and antimicrobial properties [2-6].

Chitosan is typically produced through the deacetylation of chitin found in crustacean waste, shellfish, and microbial cell walls [7-8]. Encapsulation of chitosan with other materials can also improve the water solubility of carotenoid compounds and eliminate undesirable tastes caused by environmental factors [9-10]. Chitosan has also been used as a food additive in countries such as China, Japan, Asia, and Korea [2] and as a coating for various foods. On the other hand, shrimp processing industries produce a large amount of waste that contains bioactive compounds such as chitin, which can be used in the production of chitosan for various food and feed ingredients [11-12]. Chitosan nanoparticles can also be produced through the Ionic gelation technique from chitosan and can be used to extend the shelf-life of fruits and for food packaging properties [13-14]. For example, chitosan nanoparticles coated with *Satureja hortensis* essential oil have been found to extend the shelf-life and stability of pomegranate arils [15-16]. However, chitosan nanoparticles have not been widely explored in food additives, packaging, and shelf-life extension compared to chitosan, which limits its potential applications in the food industry. Most studies have focused on the preservation of orange and apple juices [17-18].

Response Surface Methodology (RSM) is a statistical technique employed to enhance outcomes by examining the relationship between independent factors (predictor variables) and dependent variables (responses). RSM enables the optimization of responses by either maximizing or minimizing them. The aim is to pinpoint stationary positions where the partial derivatives equate to zero [19]. These stationary points may manifest as a maximum, minimum, or saddle point. The methodology involves creating models that accurately represent real-world scenarios while accommodating uncertainties in both the models and parameter values [20].

In our research, we have synthesized chitosan and chitosan nanoparticles for the preservation of sweet lime juice. We have used central composite design experiments (RSM) to optimize the pH, turbidity, and aerobic counts in the preservation of the fruit juice using chitosan and chitosan nanoparticles.

2. Materials and methods

2.1 *Preparation of chitosan and chitosan nanoparticle*

The chitosan used in this study was commercially purchased from India Sea Food, Cochin, India. The chitosan nanoparticles were synthesized from the purchased chitosan through the Ionic gelation method [15]. This process involves the ionic interaction of chitosan in acetic acid to create a chitosan emulsion. The emulsion is then dissolved and titrated with anionic Sodium tripolyphosphate dissolved in distilled water. The mixture is mixed using a magnetic stirrer, and the final product, a microgel, is frozen at 4 °C for 24 h, filtered, and dried at 60 °C. The final product is then powdered using a mortar and pestle.

2.2 *Preparation of sweet lime juice*

The sweet lime fruits were collected, peeled, and then the juice was extracted using a juicer. The juice was then filtered to remove any pulp.

2.3 *pH*

The pH of the sweet lime juice was measured and the reading taken on the first day was recorded as the initial value.

2.4 *Turbidity*

The turbidity of the sweet lime juice was determined by diluting the juice with distilled water in a ratio of 1:25 and the level of light absorption was measured using a Ultraviolet-Visible (UV-Vis) spectrophotometer at 810 nm.

2.5 *Aerobic count*

The aerobic count of the fresh sweet lime juice was determined by the spread plate method. In this method, 1 mL of juice was spread over a plate containing 20 mL of nutrient agar medium. The plates were then incubated at 37 °C for 24 h and the number of colonies formed was counted as the aerobic count.

2.6 *Fruit juice preservation*

The sweet lime juice was divided into 10 different conical flasks, in which different concentrations of chitosan (0.5 g, 1.0 g, 1.5 g, 2.0 g) and chitosan nanoparticles (0.5 g, 1.0 g, 1.5 g, 2.0 g) were added to the juice. The juice with chitosan and chitosan nanoparticles, as well as a control sample without any additives, were monitored daily for pH, turbidity, and aerobic count.

Table 1. Central composite design for the two independent variables on maximum pH, turbidity, and aerobic counts in actual and predicted values

Storage days	Concentration of chitosan (g/L)	pH		Turbidity		Aerobic counts	
		Actual value	Predicted value	Actual value	Predicted value	Actual value	Predicted value
1	1	4.586	4.537	0.331	0.334	0	0
7	1	4.479	4.478	0.304	0.302	232	246.979
1	3	4.479	4.451	0.356	0.355	0	0
7	3	4.214	4.234	0.290	0.284	168	148.863
0.432379	1.5	4.420	4.481	0.343	0.342	0	0
7.567621	1.5	4.339	4.318	0.275	0.281	201	205.656
4	0.810793	4.560	4.598	0.306	0.305	168	133.178
4	3.189207	4.399	4.402	0.301	0.307	32	54.553
4	1.5	4.515	4.511	0.285	0.284	68	69.437
4	1.5	4.515	4.511	0.285	0.284	68	69.437
4	1.5	4.515	4.511	0.285	0.284	68	69.437
4	1.5	4.515	4.511	0.285	0.284	68	69.437
4	1.5	4.515	4.511	0.285	0.284	68	69.437

2.7 *Selection of significant variables*

The selection of significant variables for optimizing pH, turbidity, and aerobic counts for different concentrations of chitosan and chitosan nanoparticles (g/L) and storage days were tested and identified through central composite design experiments. The experiment tested different combinations of chitosan and chitosan nanoparticles (1.0, 1.5, 3.0 g/L) and storage days (1.0, 4.0, and 7.0) at different parameters of the above variables (Tables 1 and 2). The statistical software package Design Expert 7.0.1, United States was used to analyze the parameters (pH, turbidity, and aerobic counts).

Table 2. Central composite design for the two independent variables on maximum pH, turbidity, and aerobic counts in actual and predicted values

Storage days	Concentration of chitosan nanoparticle (g/L)	pH		Turbidity		Aerobic counts	
		Actual value	Predicted value	Actual value	Predicted value	Actual value	Predicted value
1	1	4.41	4.375	0.136	0.155	128	149.798
7	1	4.38	4.417	0.243	0.273	368	361.744
1	3	4.41	4.389	0.136	0.141	128	145.572
7	3	4.68	4.732	0.323	0.338	232	221.518
0.432379	1.5	4.43	4.479	0.138	0.122	124	92.408
7.567621	1.5	4.78	4.707	0.343	0.309	248	263.589
4	0.810793	4.48	4.479	0.278	0.241	289	277.445
4	3.189207	4.7	4.676	0.284	0.272	196	191.552
4	1.5	4.84	4.843	0.254	0.259	172	173.875
4	1.5	4.84	4.843	0.254	0.259	172	173.875
4	1.5	4.84	4.843	0.254	0.259	172	173.875
4	1.5	4.84	4.843	0.254	0.259	172	173.875
4	1.5	4.84	4.843	0.254	0.259	172	173.875

3. Results

Figure 1 displays the results of High-Resolution Scanning Electron Microscopy (HR-SEM) conducted on chitosan and chitosan nanoparticles. The HR-SEM images reveal that while chitosan appears agglomerated, chitosan nanoparticles exhibit a distinct rod-like morphology, highlighting their promising attributes for prolonging the shelf life of food products. Research has shown that chitosan nanoparticles have been explored for their efficacy in preserving juices. For instance, incorporating chitosan into mango juice, has demonstrated an extension of the juice's shelf life by up to ten days. This study underscores the potential of chitosan nanoparticles as a viable and safe natural preservative for juices, surpassing the efficacy of pristine chitosan [21]. The average particle sizes of chitosan and chitosan nanoparticles are 33.53 nm and 23.24 nm, respectively, consistent with findings from previous research [22-23]. Decreasing the particle size leads to an increase in surface area, a factor that can improve the preservation of fruit juice.

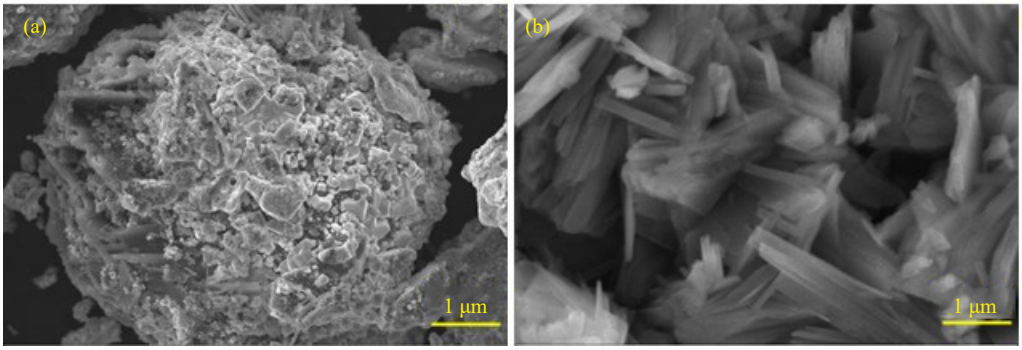


Figure 1. HR-SEM image of (a) chitosan (b) chitosan nanoparticle

3.1 *Chitosan*

The Analysis of Variance (ANOVA) analysis for the optimization study indicated that the model terms, for pH activity linear $PX_1 < 0.009$, $PX_2 < 0.0018$, and quadratic $PX_1^2 < 0.0030$, were significant ($p < 0.05$). Turbidity activity linear $PX_1 < 0.0001$, $PX_2 < 0.0001$, quadratic $PX_2^2 < 0.0001$, $PX_3^2 < 0.0001$, were significant ($p < 0.05$) and Aerobic counts activity linear $PX_1 < 0.001$, $PX_2 < 0.0059$ were significant ($p < 0.05$). Other interaction terms were neglected. Data analysis using the statgraphics software at 92% of confidence level permitted to obtain a semi-empirical expression consists of 13 statistically significant coefficients having an absolute value greater than zero, with a probability of 95% ($p < 0.05$). The results of the ANOVA indicate that the predictability of the model is at 92% and 95% confidence intervals (Tables 3-5).

Table 3. The central composite design for the two independent variables on maximum biomass activity in actual and predicted values. pH-chitosan

Source	Sum of squares	df	Mean square	F value	*p*-value prob > F
Model	0.112699	5	0.02254	16.3444	0.0009
A-storage days	0.032282	1	0.032282	23.82397	0.0018
B-concentration	0.046512	1	0.046512	34.32578	0.0006
AB	0.006253	1	0.006253	4.61483	0.0688
A^2	0.026696	1	0.026696	19.70169	0.0030
B^2	0.00024	1	0.00024	0.177122	0.6865
Residual	0.009485	7	0.001355	-	-
Lack of fit	0.009485	3	0.003162	-	-
Pure error	0	4	0	-	-
Cor total	0.122184	12	-	-	-

Table 4. The central composite design for the two independent variables on maximum biomass activity in actual and predicted values. Turbidity-chitosan

Source	Sum of squares	df	Mean square	F value	*p*-value prob > F
Model	0.007645	5	0.001529	93.29408	< 0.0001
A-storage days	0.004449	1	0.004449	271.4805	< 0.0001
B-concentration	3.66	1	3.66E-06	0.22346	0.6508
AB	0.00038	1	0.00038	23.2027	0.0019
A^2	0.001566	1	0.001566	95.5435	< 0.0001
B^2	0.000988	1	0.000988	60.30006	0.0001
Residual	0.000115	7	1.64	-	-
Lack of fit	0.000115	3	3.82	-	-
Pure error	0	4	0	-	-
Cor total	0.007759	12	-	-	-

Table 5. The central composite design for the two independent variables on maximum biomass activity in actual and predicted values. Aerobic counts-chitosan

Source	Sum of squares	df	Mean square	F value	*p*-value prob > F
Model	71,228.84048	5	14,245.77	28.95929324	0.0002
A-storage days	59,802.95883	1	59,802.96	121.5695363	< 0.0001
B-concentration	7,462.188668	1	7,462.189	15.16939686	0.0059
AB	1,024	1	1,024	2.081622842	0.1923
A^2	1,359.217181	1	1,359.217	2.763063996	0.1404
B^2	1,305.238791	1	1,305.239	2.653334847	0.1474
Residual	3,443.467209	7	491.9239	-	-
Lack of fit	3,443.467209	3	1,147.822	-	-
Pure error	0	4	0	-	-
Cor total	74,672.30769	12	-	-	-

3.2 *Chitosan nanoparticle*

The ANOVA analysis of the optimization study indicated that the model terms, for pH activity linear $PX_1 < 0.008$, $PX_2 < 0.0018$, quadratic linear $PX_2^2 < 0.0001$, turbidity activity linear $PX_1 < 0.0001$, aerobic counts activity linear $PX_1 < 0.0001$, $PX_2 < 0.0001$, and quadratic $PX_2^2 < 0.0018$ were significant ($p < 0.05$). Other interaction terms were neglected. Data analysis using the statgraphics software at 96% of confidence level permitted to obtain a semi-empirical expression which consists of 13 statistically significant coefficients having an absolute value greater than zero, with a probability of 96% ($p < 0.05$). The goodness of fit of the regression model can be ascertained by applying the F-test which explains perfectly the experimental range studied. The results of the ANOVA indicate that the predictability of the model is at

92% and 96% confidence intervals (Tables 6-8).

Table 6. The central composite design for the two independent variables on coefficient (ANOVA) in actual and predicted values. pH-chitosan nanoparticle

Source	Sum of squares	df	Mean square	F value	*p*-value prob > F
Model	0.452143	5	0.090429	45.12896	< 0.0001
A-storage days	0.063064	1	0.063064	31.4725	0.0008
B-concentration	0.046193	1	0.046193	23.05275	0.0020
AB	0.0225	1	0.0225	11.22877	0.0122
A^2	0.136763	1	0.136763	68.25242	< 0.0001
B^2	0.153663	1	0.153663	76.6866	< 0.0001
Residual	0.014026	7	0.002004	-	-
Lack of fit	0.014026	3	0.004675	-	-
Pure error	0	4	0	-	-
Cor total	0.466169	12	-	-	-

Table 7. The central composite design for the two independent variables on coefficient (ANOVA) in actual and predicted values. Turbidity-chitosan nanoparticle

Source	Sum of squares	df	Mean square	F value	*p*-value prob > F
Model	0.049332	5	0.009866	15.3572	0.0012
A-storage days	0.042355	1	0.042355	65.92576	< 0.0001
B-concentration	0.001112	1	0.001112	1.730699	0.2298
AB	0.0016	1	0.0016	2.490431	0.1585
A^2	0.004152	1	0.004152	6.463435	0.0385
B^2	2.06	1	2.06	0.032117	0.8628
Residual	0.004497	7	0.000642	-	-
Lack of fit	0.004497	3	0.001499	-	-
Pure error	0	4	0	-	-
Cor total	0.053829	12	-	-	-

3.3 *Effect of pH*

The pH activity was plotted against chitosan concentrations in the range of 1.0-3.0 g/L and storage days in the range of 1.0-7.0. At chitosan concentrations of 1.0-1.5 g/L and storage days of 1.0-5.5, the pH was 4.51 as shown in Figure 2(a). The optimum level of pH activity was calculated to be 92% at 1.5 g/L of chitosan and 4.5 storage days.

Similarly, for chitosan nanoparticles, the pH was in the range of 1.0-3.0 g/L with respect to the storage days of 1.0-7.0. The pH of 4.84 was observed in the chitosan nanoparticles of 1.5-2.5 g/L and storage days of 4.0-5.5 as shown in Figure 2(b). The optimum level of pH activity was calculated to be 92% at the chitosan nanoparticles of 1.5 g/L and storage days of 4.0. The obtained pH range was between 3.0-4.6 for chitosan and 3.0-4.9 for chitosan nanoparticles.

Table 8. The central composite design for two independent variables on coefficient (ANOVA in actual and predicted values). Aerobic counts-chitosan nanoparticle

Source	Sum of squares	df	Mean square	F value	*p*-value prob > F
Model	57,151.99	5	11,430.4	34.12161	< 0.000
A-storage days	35,371.92	1	35,371.92	105.591	< 0.0001
B-concentration	8,905.377	1	8,905.377	26.58401	0.0013
AB	4,624	1	4,624	13.8034	0.0075
A^2	37.19155	1	37.19155	0.111023	0.7487
B^2	8,038.746	1	8,038.746	23.99697	0.0018
Residual	2,344.93	7	334.99	-	-
Lack of fit	2,344.93	3	781.6434	-	-
Pure error	0	4	0	-	-
Cor total	59,496.92	12	-	-	-

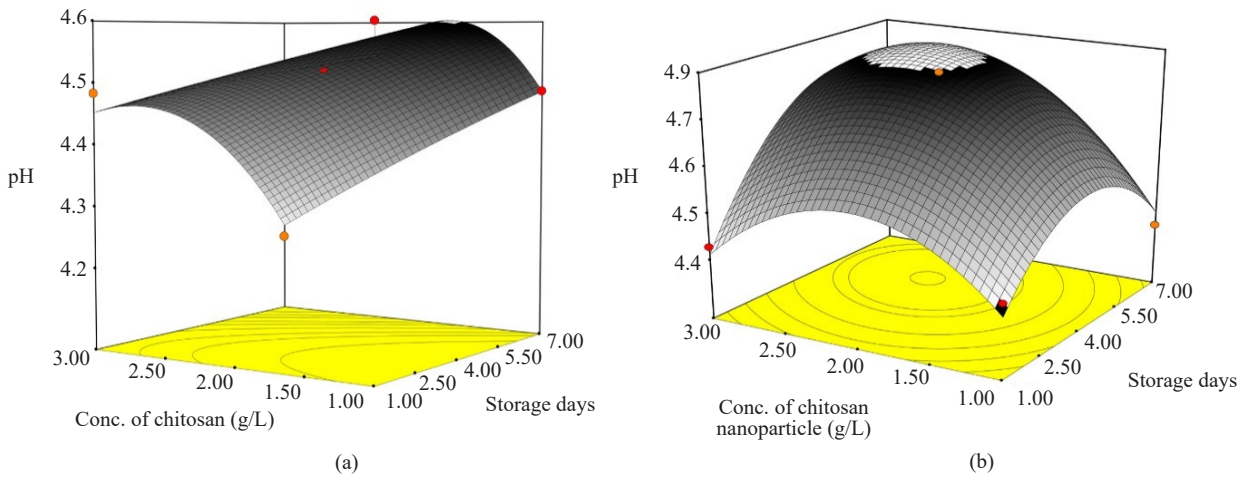


Figure 2. 3D plot representing pH activity of concentration of (a) chitosan and (b) chitosan nanoparticle with storage days

3.4 *Effect of turbidity*

The turbidity activity was observed in the chitosan concentration of 1.5-2.5 g/L and storage days (4.0 to 7.0) at the level of turbidity activity (92%) was shown in Figure 3(a). The optimum level of chitosan (0.5-2.5 g/L) and storage days (1.0-2.0) displayed the maximum turbidity activity at 0.284 g/L respectively. Increasing the chitosan from 1.0-3.0 g/L has increased the turbidity activity from 1.5-2.5 g/L. Similarly, for chitosan nanoparticles, the turbidity activity

(92%) was observed in the chitosan nanoparticles (1.0-3.0 g/L) and storage days (4.0-7.0) as shown in Figure 3(b). The optimum level of chitosan nanoparticles (1.5-2.5 g/L) and storage days (4.0-5.0) showed the maximum turbidity activity at 0.254 g/L. Increasing the chitosan nanoparticles from 1.5-3.0 g/L has increased the turbidity activity from 0-0.275 g/L.

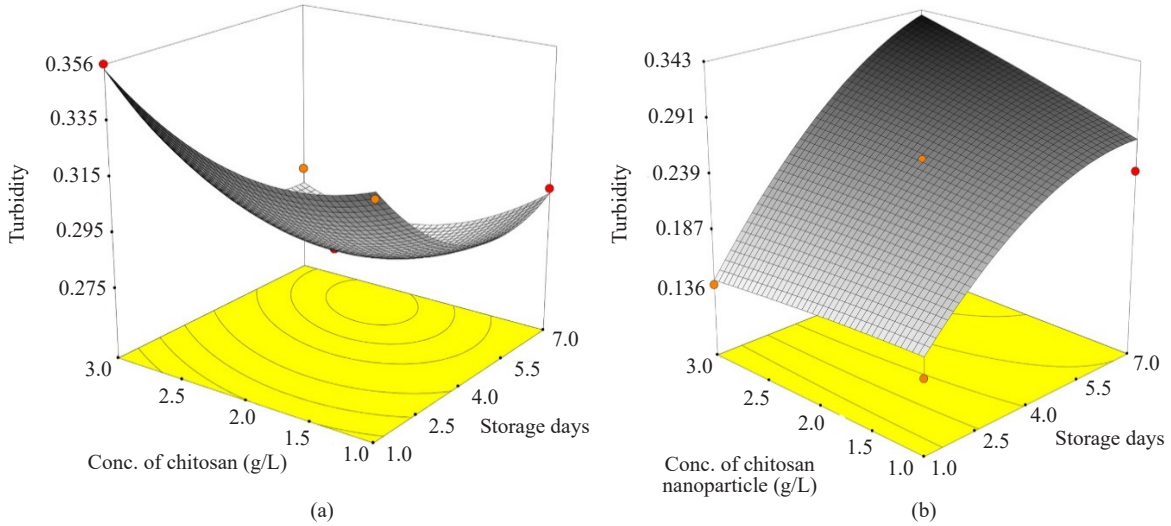


Figure 3. 3D plot representing turbidity of concentration of (a) chitosan and (b) chitosan nanoparticle with storage days

3.5 *Effect of aerobic counts (CFU)*

Aerobic counts against the chitosan concentrations (1.5-3.0 g/L) and storage days (1.0-2.5) are shown in Figure 4(a). The aerobic counts (69.44 g/L) at the chitosan (1.5-3.0 g/L) and storage days (0-1.2) depict the optimum level of aerobic counts activity occurs with 92% at the chitosan of 2.0 g/L and storage days at 1.0 calculated by derivation. Similarly, for chitosan nanoparticles, the aerobic counts against chitosan nanoparticles (1.5-3.0 g/L) and storage days (1.0-2.5) are shown in Figure 4(b). The aerobic counts (173.87 g/L) at the chitosan nanoparticles (1.5-3.0 g/L) and storage days (0-1.2) depict the optimum level of aerobic counts occurs with 92% at the chitosan nanoparticles 2.0 g/L and storage days at 1.0.

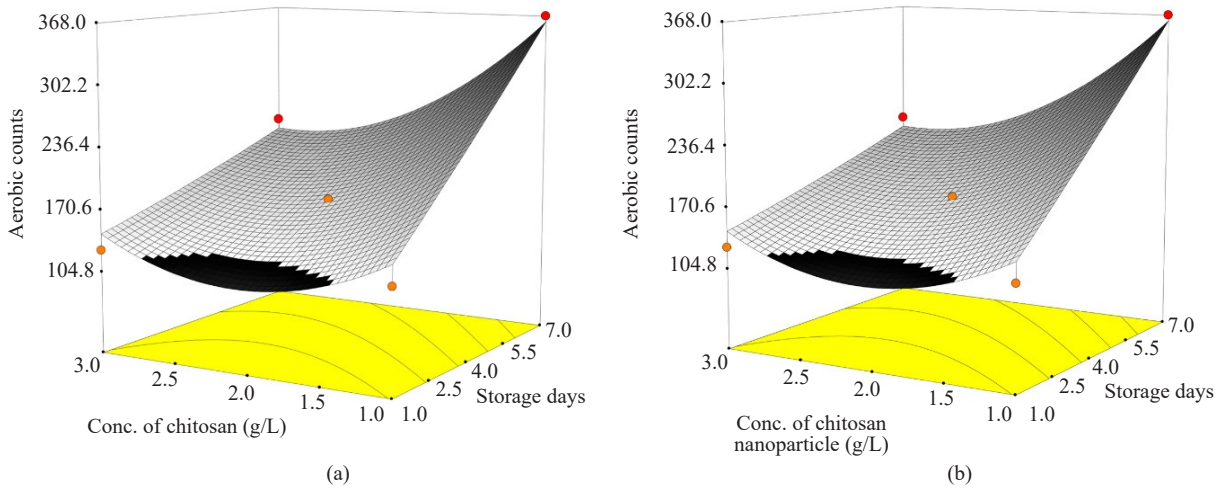


Figure 4. 3D plot representing aerobic counts of concentration of (a) chitosan and (b) chitosan nanoparticle with storage days

Various food preservation materials, such as alginate, sesame, *Solidago canadensis* L., and chitosan-based coatings, have been investigated for their effectiveness in extending the shelf life and preserving the quality of fruits and juices. Alginate coatings containing 1.0% of sodium alginate have been shown to create uniform and sturdy membranes, preserving the quality of pitaya fruit and sweet cherries for extended periods [24]. Additionally, coatings made from sesame have demonstrated efficacy in protecting orange juice from microbial spoilage [25]. *Solidago canadensis* L. coatings have been found to maintain the post-harvest quality of strawberries, while chitosan-based coatings, particularly those incorporating lemon essential oil, have shown enhanced preservation against fungal decay in strawberries [26]. Furthermore, chitosan nanoparticle coatings crosslinked with sodium tripolyphosphate have been effective in preserving sweet lime juice for up to 7 days. These findings underscore the potential of various coating materials and crosslinking agents in prolonging the shelf life and enhancing the quality of fruits and juices. Table 9 shows the comparison of chitosan and chitosan nanoparticle with other food preservation materials.

Table 9. Comparison of chitosan and chitosan nanoparticle with other food preservation materials

Food preservation materials	Coating/crosslinking	Fruit juice preserved	Results	References
Alginate	1.0% of sodium alginate	Pitaya	pH ranged from 4.0 to 7.0 facilitates the creation of membranes that are uniform and sturdy, while also reducing the transmission of H+ ions and soluble particles across the capsules.	[24]
Alginate	Cherries	Sweet cherry	Cherries coated with alginate maintained optimal quality and increased antioxidant activity for up to 16 days at 2 °C, followed by an additional 2 days at 20 °C.	[25]
Sesame (*Sesamum indicum*)	-	Orange juice	Preserved orange juice from microbial spoilage.	[26]
Solidago canadensis L.	-	Strawberry fruit and juice	Preserved the post-harvest quality of strawberries.	[27]
Chitosan-lemon essential oil	Chitosan	Strawberry	Enhanced fruit preservation against fungal decay, particularly with the inclusion of lemon essential oil in the coating.	[28]
Chitosan nanoparticle	Sodium tripolyphosphate	Sweet lime	Preserved the juice for 7 days.	Present work

4. Discussion

The objective of this research was to develop a quadratic model with 39 trials. The model was designed by central composite to study the effects of chitosan concentration of 1.0, 2.0, 3.0 (g/L) with storage days (1.0, 4.0, 7.0) using the predicted values (Table 1). The F-test (Tables 3-5) with a very low probability value (P model > F = 0.0001) proves a very high significance for the regression model. The determination coefficient (R^2) was used to evaluate the goodness of fit of the model. The goodness of fit of the regression model can be ascertained by applying the F-test. This model explains perfectly the experimental range studied [29]. The Model F-value of 16.96 (pH); Model F-value of (turbidity) 93.29; Model F-value of 28.96 (Aerobic counts) implies the model is significant. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, A, B, AB, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The (pH); "Pred R-Squared" of 0.9223 is in reasonable agreement with the "Adj R-Squared" of 0.8669; the (turbidity); "Pred R-Squared" of 0.9852 is in reasonable agreement with the "Adj R-Squared" of 0.9746; the (aerobic counts); "Pred R-Squared" of 0.9538 is in reasonable agreement with the "Adj R-Squared" of 0.9209.

The central composite design experiments were used to determine chitosan nanoparticle concentration of 1.0, 2.0, 3.0 (g/L) with storage days (1.0, 4.0, 7.0), which are depicted in Table 2, along with the predicted values. The F-test (Tables 6-8) with a very low probability value (P model > F = 0.0001) demonstrated a very high significance for the regression model where the determination coefficient (R^2) was used to evaluate the goodness of fit of the model. The Model F-value of 45.13 (pH); Model F-value of (turbidity) 15.36; Model F-value of 34.12 (Aerobic counts) implies that the model is significant. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, A, B, AB, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The (pH); "Pred R-Squared" of 0.9699 is in reasonable agreement with the "Adj R-Squared" of 0.9484; the (turbidity); "Pred R-Squared" of 0.9164 is in reasonable agreement with the "Adj R-Squared" of 0.8567; the (aerobic counts); "Pred R-Squared" of 0.9605 is in reasonable agreement with the "Adj R-Squared" of 0.9324.

The results of the study showed that increasing the initial chitosan concentration not only increases the turbidity activity of the sweet lime juice, but also the storage days required for the completion of the turbidity activity. This was observed in a study on the quality attributes of sweet lime juice subjected to different storage conditions [30]. The research highlighted the impact of storage temperature and packaging material on the quality of ultrasound-treated sweet lime juice [31]. Additionally, another study discussed how citrus scion/rootstock combinations affect the concentration of bioactive compounds in orange juice and emphasized that packaging and storage can maximize the shelf life of freshly squeezed juice [32]. Without any coatings, chitosan nanoparticle itself increased the storage days, exhibiting biocompatibility [25]. The study also revealed that chitosan concentration played a significant role in controlling the turbidity of the juice. The storage days were found to have a significant effect on the juice's turbidity ($p < 0.05$). Additionally, lower concentrations of chitosan were found to produce high-turbidity juices when compared to higher chitosan concentrations. The positive reductions observed in this study suggest the potential use of chitosan and chitosan nanoparticle in preserving the sweet lime juice.

5. Conclusion

This research study aimed to investigate the use of chitosan and chitosan nanoparticles as natural preservatives for sweet lime juice. The study found that chitosan and chitosan nanoparticles have the ability to extend the shelf life of the juice by maintaining pH, turbidity, and aerobic count at optimal levels. The research utilized a response surface methodology to optimize these parameters through the use of central composite design experiments. The results of the study showed that a high concentration of chitosan and chitosan nanoparticle was more effective in maintaining the quality of the juice compared to low concentrations. The study suggests that chitosan and chitosan nanoparticles could be used as a natural preservative for fruit juice preservation and further research on the effect of deacetylation degree and microbiological studies is recommended.

Data availability

The data that supports the findings of this study will be provided from the corresponding author on reasonable request.

Funding statement

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that there is no conflict of interest.

References

[1] CDC. *Foodborne Illnesses and Germs*. Centers for Disease Control and Prevention. 2018. Available from: https://www.cdc.gov/foodsafety/foodborne-germs.html [Accessed 16th April 2024].

[2] Butnaru E, Stoleru E, Brebu MA, Darie-Nita RN, Bargan A, Vasile C. Chitosan-based bionanocomposite films prepared by emulsion technique for food preservation. *Materials (Basel)*. 2019; 12(3): 373.

[3] Matica MA, Aachmann FL, Tøndervik A, Sletta H, Ostafe V. Chitosan as a wound dressing starting material: Antimicrobial properties and mode of action. *International Journal of Molecular Sciences*. 2019; 20(23): 5889.

[4] Arafat Y, Altemimi A, Ibrahim SA, Badwaik LS. Valorization of sweet lime peel for the extraction of essential oil by solvent free microwave extraction enhanced with ultrasound pretreatment. *Molecules*. 2020; 25(18): 4072.

[5] Zheng L-Y, Zhu J-F. Study on antimicrobial activity of chitosan with different molecular weights. *Carbohydrate Polymers*. 2003; 54(4): 527-530.

[6] Yalpani M, Johnson F, Robinson LE. Antimicrobial activity of some chitosan derivatives. *Advances in Chitin and Chitosan*. 1992; 543-548.

[7] Muthu M, Gopal J, Chun S, Devadoss AJP, Hasan N, Sivanesan I. Crustacean waste-derived chitosan: Antioxidant properties and future perspective. *Antioxidants (Basel)*. 2021; 10(2): 228.

[8] Hafsa J, Smach MA, Sobeh M, Majdoub H, Yasri A. Antioxidant activity improvement of apples juice supplemented with chitosan-galactose maillard reaction products. *Molecules*. 2019; 24(24): 4557.

[9] Rocha MAM, Coimbra MA, Nunes C. Applications of chitosan and their derivatives in beverages: a critical review. *Current Opinion in Food Science*. 2017; 15: 61-69.

[10] Helander IM, Nurmiaho-Lassila EL, Ahvenainen R, Rhoades J, Roller S. Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. *International Journal of Food Microbiology*. 2001; 71(2-3): 235-244.

[11] Nirmal NP, Santivarangkna C, Rajput MS, Benjakul S. Trends in shrimp processing waste utilization: An industrial prospective. *Trends in Food Science & Technology*. 2020; 103: 20-35.

[12] Chatterjee S, Chatterjee S, Chatterjee BP, Guha AK. Clarification of fruit juice with chitosan. *Process Biochemistry*. 2004; 39(12): 2229-2232.

[13] Rungsardthong V, Wongvuttanakul N, Kongpien N, Chotiwaranon P. Application of fungal chitosan for clarification of apple juice. *Process Biochemistry*. 2006; 41(3): 589-593.

[14] Shahidi F, Arachchi JKV, Jeon YJ. Food applications of chitin and chitosans. *Trends in Food Science & Technology*. 1999; 10(2): 37-51.

[15] Amiri A, Ramezanian A, Mortazavi SMH, Hosseini SMH, Yahia E. Shelf-life extension of pomegranate arils using chitosan nanoparticles loaded with *Satureja hortensis* essential oil. *Journal of the Science of Food and Agriculture*. 2020; 101(9): 3778-3786.

[16] Kisko G, Sharp R, Roller S. Chitosan inactivates spoilage yeasts but enhances survival of *Escherichia coli* O_{157}: H_7 in apple juice. *Journal of Applied Microbiology*. 2005; 98(4): 872-880.

[17] Wu D, Zhang M, Xu B, Guo Z. Fresh-cut orange preservation based on nano-zinc oxide combined with pressurized argon treatment. *LWT-Food Science and Technology*. 2021; 135: 110036. Available from: doi: 10.1016/j.lwt.2020.110036.

[18] Sportelli MC, Ancona A, Volpe A, Gaudiuso C, Lavicita V, Miceli V, et al. A new nanocomposite packaging based on LASiS-Generated AgNPs for the preservation of apple juice. *Antibiotics*. 2021; 10(7): 760.

[19] Khuri AI, Mukhopadhyay S. Response surface methodology. *WIREs Computational Statistics*. 2010; 2(2): 128-149.

[20] Panwar V, Kumar Sharma D, Pradeep Kumar KV, Jain A. Experimental investigations and optimization of surface roughness in turning of en 36 alloy steel using response surface methodology and genetic algorithm. *Materials Today Proceedings*. 2021; 46: 6474-6481.

[21] Mahadik N, Rajesh P. *Biosynthesis of chitosan mediated nanoparticles for preservation of food and its antimicrobial activity*. Semantic Scholar. 2017. Available from: https://api.semanticscholar.org/CorpusID:226930641 [Accessed 16th April 2024].

[22] Khanmohammadi M, Elmizadeh H, Ghasemi K. Investigation of size and morphology of chitosan nanoparticles used in drug delivery system employing chemometric technique. *Iranian Journal of Pharmaceutical Research*. 2015; 14(3): 665-675.
[23] Jeyaprakash JS, Murugan P, Balu SK, Annadurai G. Revealing the in vitro cytotoxicity potential of chitosan-mediated SiO_2/ZnO nanocomposites on the human MCF-7 cell line. *Emergent Materials*. 2023; 6: 1577-1587.
[24] Rodríguez-Sánchez JA, Cuatzo-Lozano MI, Pérez-Loredo MG, Abarca-Sarro DI, Navarro YG. Alginate encapsulation as a preservation method of pitaya fruit juice (*Stenocereus* spp.). *Journal of Food Science and Engineering*. 2017; 7: 127-134.
[25] Díaz-Mula HM, Serrano M, Valero D. Alginate coatings preserve fruit quality and bioactive compounds during storage of sweet cherry fruit. *Food and Bioprocess Technology*. 2012; 5: 2990-2997.
[26] Ghosh V, Mukherjee A, Chandrasekaran N. Eugenol-loaded antimicrobial nanoemulsion preserves fruit juice against, microbial spoilage. *Colloids and Surfaces B: Biointerfaces*. 2014; 114: 392-397.
[27] Liu S, Shao X, Wei Y, Li Y, Xu F, Wang H. *Solidago canadensis* L. essential oil vapor effectively inhibits botrytis cinerea growth and preserves postharvest quality of strawberry as a food model system. *Frontiers in Microbiology*. 2016; 7: 01179.
[28] Perdones A, Sánchez-González L, Chiralt A, Vargas M. Effect of chitosan-lemon essential oil coatings on storage-keeping quality of strawberry. *Postharvest Biology and Technology*. 2012; 70: 32-41.
[29] Cochran WG, Cox GM. *Experimental Designs*. New York: John Wiley & Sons; 1992.
[30] Shaik L, Chakraborty S. Effect of different storage conditions on the quality attributes of sweet lime juice subjected to pulsed light and thermal pasteurization. *Sustainable Food Technology*. 2023; 1(5): 722-737.
[31] Shaik L, Chakraborty S. The impact of storage temperature and packaging material on the quality of ultrasound treated sweet lime juice. *Food and Humanity*. 2023; 1: 445-458.
[32] Martins RC, Leonel S, Souza JMA, Lima GPP, Leonel M, Putti FF, et al. Profile of bioactive compounds in orange juice related to the combination of different scion/rootstocks, packaging and storage. *Horticulturae*. 2023; 9(3): 347.