A model for predicting microbiological and organoleptic indicators of salads during storage with the use of chitosan

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Abstract. The authors theoretically substantiated and experimentally confirmed the feasibility of using high-molecular acid-soluble chitosan (HAC), which inhibits the growth of microbial flora in the production of salads, to extend the expiration date of salads and ensure the quality and safety indicators of products during storage. The effect of «cold pasteurization» of HAC on the microflora of salads was established. Regression models were obtained that adequately describe the effect of HAC on the microbiological and organoleptic indicators of salads. A method for multi-criteria optimization of the technological mode of production of salads using chitosan according to microbiological and organoleptic indicators to extend their expiration date is proposed. A method for the introduction of HAC in the production of salads in the composition of the dressing – in the form of a 1 % solution in 3 % acetic acid. Salad dressing recipe using HAC was developed. The efficiency of the production technology of salads with the use of HAC was confirmed, which allows one to extend their expiration date at a storage temperature of (4 ± 2) °C from 12 to 78 h.

1. Introduction
The relevance of monitoring and control in the field of public catering is dictated by a stable growth of the infection of the population directly related to the use of food of inadequate quality. According to WHO, up to 1-1.2 billion «diarrheal» diseases are registered annually in the world, the consequences of which can be fatal.

One of the most effective ways of suppressing pathogenic microflora and ensuring the sanitary safety of products today is the use of food preservatives. This group of food additives has both synthetic and natural origin; however, chemical preservatives that can completely inhibit the growth of microflora are in the greatest demand.

However, when there is a question about the effect of preservative on the accompanying microflora in the feedstock, as well as the safety of its impact on the human body, it is very important to take an objective approach to the choice of preservative and its dosage in the production of food.

Considering the increase in the number of resistant strains of pathogenic microflora, it can be concluded that widely used preservatives are not able to effectively suppress the vital activity of...
microorganisms circulating in food products and the working environment having antibiotic resistance and pathogenicity factors.

The search for new antibacterial and antymycotic substances currently remains an urgent task in connection with the constant emergence of polyresistant to classical antibiotics and antymycotics strains of conditionally pathogenic microorganisms.

At present time, as preservatives are becoming popular substances of natural origin with antimicrobial properties, one of which are bacteriocytes, having a pronounced antimicrobial action against a wide range of microorganisms.

One of the most promising antibacterial agents is chitosan, a unique natural biopolymer with an extensive spectrum of biological activity.

Chitosan belongs to the 4th class of toxicity and is considered safe. It was determined that for chitosan LD50 = 16 g/kg of the weight of mice (for comparison, in sugar LD50 = 12 g/kg).

One of the first properties found in chitosan was its antibacterial and anti-fungal activity. It has been established that chitosan exhibits antibacterial activity against both gram-negative and gram-positive bacteria [1-4].

It is assumed that the mechanism of the antibacterial and antifungal action of chitosan is associated with its ability to bind with negatively charged cell surface structures. Such interaction disrupts the normal functioning of the metabolic processes of the cell with the external environment, changes in the permeability of the cytoplasmic membrane, as a result of which the outflow of substances from the cell increases [1-4].

The antimicrobial effect is most dependent on the concentration of chitosan (rectilinear dependence) and, to a lesser extent, on its phase-aggregate state – solution, gel, or solid-phase material [1-4].

However, in food production, chitosan solutions are mainly used with the use of various solvents depending on the type of chitosan polymer.

The problem of microbiological safety is particularly acute for manufacturers of finished culinary products sold in large volumes in retail chains. Salads and other cold dishes and snacks are not subjected to heat treatment, therefore, without the use of preservative agents that do not meet the principles of healthy nutrition, have a limited expiration date.

Solving this problem is an important task for both producers interested in making a profit from their products and for consumers who want to get a high-quality and safe food product. An effective solution to the issues of safety and prolongation of the expiration date of salads is «cold pasteurization», involving the use of «barriers» (competing microflora, food additives of natural origin, bacteriocins, etc.), which slow down the growth and reproduction of microorganisms.

Taking into account the above, we conducted studies to substantiate the use of chitosan as a bio-preservative in the production of vegetable salads in order to prolong their expiration date.

2. Materials and methods

The objects of research are selected salads that are in high demand in retail chains and at catering establishments – fresh tomato and cucumber salad, and vegetable vinaigrette, differing in their different prescription composition and methods of technological preparation of products, which allows us to make an overall picture of the impact effects of the «barrier» on the presented group of dishes.

Based on the results of a preliminary analysis of the determination of the antibiotic activity of various chitosan samples (acid-soluble, water-soluble and chitosan succinate), selected high-molecular acid-soluble food chitosan (HAC), produced by Bioprogress CJSC (according to TS 9289-067-00472127-03), was shown to be effective against microbial contaminants (Escherichia coli K12, Staphylococcus aureus ATCC 6538 – PFDA 209-P, Pseudomonas aeruginosa 453, Salmonella enterica spp. Enterica serovar Enteritidis 11272, Enterococcus faecalis ATCC 29212) [5, 6].

Due to the fact that the effectiveness of preservatives depends on their concentration in the medium, in the course of studies HAC was introduced into a dressing for salads (hereinafter referred to as dressing) (table 1) as solutions in 3 % acetic acid, varying its concentration [5, 6].
Table 1. The basic recipe of a dressing for salads

| The name of prescription components | Raw material consumption, % |
|------------------------------------|-----------------------------|
| Refined deodorized vegetable oil   | 50.0                        |
| 3 % acetic acid                    | 50.0                        |
| Granulated sugar                   | 4.0                         |
| Ground black pepper                | 0.2                         |
| Table salt                         | 2.0                         |

The basis for justifying the expiration date of salads is to conduct microbiological research and assess the organoleptic properties of samples of finished products during storage.

To determine the technological mode of production of vegetable salads with a prolonged expiration date, a controlled indicator of microbiological safety, the number of mesophilic aerobic and optionally anaerobic microorganisms (NMAOAnM) has been taken. This indicator characterizes the total content of microorganisms in the product, is used everywhere to assess the quality of the finished product, with the exception of those in the production of which special microbial cultures are used.

Before processing, the samples were portioned 10 g each into separate sealed plastic boxes to prevent secondary contamination during material sampling.

Samples were stored until seeding at a temperature of (4 ± 2) °C. The first seeding of the samples was carried out at the beginning of storage (background) to determine the initial contamination of the samples (P₀). Then the crops were made during storage of the samples studied every 24 h for 120 h.

Selection and preparation of samples, the procedure for evaluating the organoleptic characteristics of salads during storage was carried out in accordance with GOST 31986-2012 «Catering services. Method of organoleptic evaluation of the quality of catering products».

The tasting evaluation of the samples was carried out by simultaneous presentation of the coded samples of the product under investigation at the end of the expected expiration date (with positive results of laboratory tests), at intervals time (24 h) and an analogous freshly produced product.

Organoleptic analysis of vegetable salads includes a rating assessment of appearance, texture (consistency), smell and taste using a point scale: 10-9 points – excellent quality, 8-7 points – good quality, 6-5 points – satisfactory quality and 4 points – unsatisfactory quality. Rating assessment of the quality of vegetable salads was carried out as a whole (overall quality level). According to the results of sensory analysis, the quality criterion of organoleptic indicators was calculated for each sample in the range from 0 to 1.0.

To ensure the statistical validity of the results, the number of independent participants of the tasting who are not aware of the sample codes is 3 people.

Experimental studies were carried out in the research laboratories of the Department of Catering and Service and the Center for Collective Use of the Research Center for Food and Chemical Technologies of the Kuban State Technological University, as well as the Department of Epizootology, Mycology and Veterinary-Sanitary Expertise of the Krasnodar Scientific and Research Veterinary Institute (separate structural unit of the Federal State Budgetary Scientific Institution «Krasna Darski Research Center for Animal Breeding and Veterinary Medicine»).

Establishing the dependence of the growth of microflora on the initial contamination of NMAOAnM salads (P₀), their storage time (t, h) and the concentration of HAC (X, %), as well as the regression analysis of the dependence of the organoleptic assessment criterion on the duration of storage of salads and concentration HAC, made in the software environment Statistica v.10. The solution of the vector optimization problem (by the criteria of maximization of the shelf life and organoleptic assessment) is performed by means of scripts of the MathCAD software environment to justify expiration dates.

Optimization of the technological mode of production of salads with the use of HAC for the extension of shelf life was conducted in accordance with the developed methodology (figure 1).
3. Results

The specification of the regression model is the initial stage of planning an experiment to develop a model for predicting the growth dynamics of the microflora of dishes $P = P(t, X)$ depending on the shelf life ($t$, h) and the concentration of HAC in the dressing ($X$, %), on the normalized food safety indicator NMAOAnM, CFU/g products, provided that the initial contamination is known (NMAOAnM) $P_0 = P(t = 0)$ of freshly prepared salad at the beginning of storage ($t = 0$ h) before introducing the dressing with HAC.

The regression model in the form of an exponential curve is:

$$P(t, X) = P_0 \cdot \exp(-aX^b) \cdot \exp([q + sX^2]t).$$

In accordance with the developed methodology (figure 1) for the optimization of the technological mode of production of salads with the use of HAC, a four-level experiment plan was constructed to extend their expiration date and microbiological studies were performed (Tables 2 and 3) of vegetable salad samples during storage in accordance with the MIN 4.2.1847.

As a result of microscopic typing, it has been established that the microflora of salads is represented mainly by spore-forming rods. Representatives of mold and yeast microflora were not found in the samples studied.
Table 2. Growth of NMAOAnM in samples of salad from fresh tomatoes and cucumbers with dressing HAC during storage

| Concentration of HAC, %, in 3 % acetic acid | Frequency of control | Shelf life, h |
|-------------------------------------------|----------------------|--------------|
|                                           | background           | 24           | 48           | 72           | 96           | 120          |
|                                           | NMAOAnM, CFU/g       |              |              |              |              |              |
| 0                                         | 1.00×10⁴             | 1.10×10⁴     | 1.00×10⁴     | 1.90×10⁴     | 2.60×10⁴     | 3.60×10⁴     |
| 0.375                                     | 0.19×10⁵             | 0.33×10⁴     | 0.25×10⁵     | 0.74×10⁴     | 1.00×10⁵     | 1.30×10⁵     |
| 0.75                                      | 8                    | 15           | 1.26×10⁴     | 0.28×10⁴     | 0.32×10⁴     | 0.50×10⁴     |
| 1.25                                      | 7                    | 14           | 0.17×10⁵     | 0.35×10⁴     | 0.44×10⁴     | 0.45×10⁴     |
| 1.50                                      | 5                    | 11           | 0.11×10⁵     | 0.20×10⁴     | 0.30×10⁴     | 0.38×10⁴     |

Due to the fact that the prescription components of the salad of fresh tomatoes and cucumbers are not a favorable medium for the reproduction of microorganisms, there is a slight increase growth of microflora in the samples (table 2).

Table 3. Growth of NMAOAnM in samples of vegetable vinaigrette with dressing HAC during storage

| Concentration of HAC, %, in 3 % acetic acid | Frequency of control | Shelf life, h |
|-------------------------------------------|----------------------|--------------|
|                                           | background           | 24           | 48           | 72           | 96           | 120          |
|                                           | NMAOAnM ×10⁴, CFU/g  |              |              |              |              |              |
| 0                                         | 0.76                 | 1.10         | 2.90         | 2.80         | 3.30         | 7.60         |
| 0.375                                     | 0.59                 | 0.41         | 0.53         | 1.00         | 1.00         | 1.00         |
| 0.75                                      | 0.48                 | 0.32         | 0.22         | 0.69         | 1.60         | 1.30         |
| 1.25                                      | 0.51                 | 0.35         | 0.22         | 0.43         | 0.45         | 0.56         |
| 1.50                                      | 0.12                 | 0.35         | 0.19         | 0.64         | 0.41         | 0.54         |

For further research, the vegetable vinaigrette was chosen, featuring a different prescription composition and methods of technological preparation of products, which allows us to create a general picture of the impact of the «barrier» on the presented group of dishes.

According to the obtained experimental data (table 3), the regression model of NMAOAnM growth was identified in the composition with the power influence of factors – the storage duration and the concentration of HAC in the salad dressing. According to experimental data, the initial contamination of vegetable vinaigrette with dressing without HAC is \( P_0 = 3.19\times10^4 \) CFU/g. The parameters of the regression model for microbiological indicators were calculated by means of the STATISTICA package. For the vegetable vinaigrette, the regression growth model of NMAOAnM from the storage time and the concentration of HAC in the dressing is (2), the correlation index is \( R = 0.97 \), and is presented in figure 2:

\[
P(t, X) = P_0 \cdot \exp(-1.541X^{0.8521}) \cdot \exp((0.012 + 0.007877X - 0.00026X^2)\epsilon)
\]  

where \( P_0 \) is the initial contamination of freshly prepared salad with dressing without HAC, CFU/g.

Experimentally confirmed the effect of "cold pasteurization" HAC in the dressing in relation to the microflora of the salad. In the course of the research, a directly proportional dependence of the decrease in the microflora of salad samples on the concentration of the introduced into the dressing of the HAC from 0 % to 1.0 % was established; a further increase in the concentration of HAC in the dressing slightly inhibits the microflora.

Considering that an important factor in justifying expiration date is the evaluation of organoleptic indicators of products during storage, a sensory analysis of samples was carried out.
The specification of the regression model of the organoleptic evaluation criterion $K_0(t, X)$ as a function of the HAC concentration in the dressing ($X, \%$) and the shelf life time ($t, h$) has the form:

$$K_0(t, X) = a_0 + c_1 X + c_2 X^2 + a_1 t + a_2 t^2 + b_{12} t X.$$  \hspace{1cm} (3)

The parameters of the regression model for organoleptic indicators calculated by means of the package STATISTICA. The regression model of the criterion for the organoleptic evaluation of $K_0(t, X)$ versus storage time ($t, h$) and the concentration of HAC in the dressing ($X, \%$) has the form (4) and is presented in figure 3:

$$K_0(t, X) = 0.89844 + 0.208695 \cdot X - 0.09714 \cdot X^2 - 0.00585 t + 0.00000103 t^2 + 0.002054 t X$$  \hspace{1cm} (4)

Figure 2. The dependence of the growth of NMAOAnM CFU/g on the shelf life of vegetable vinaigrette and the concentration of WSS in the dressing.

Despite the slowdown in the growth of microflora, the organoleptic characteristics of samples of vegetable vinaigrette are reduced during storage in relation to freshly processed products.

The decrease of the value of organoleptic characteristics of the vegetable vinaigrette samples during storage is the result of the biochemical transformations of the salad recipe components.

4. Discussions

The obtained models (2) and (4) make it possible to predict the expiration date of vegetable vinaigrette with the use of HAC in a dressing. The calculations by means of the MathCAD package are reflected in Figure 4.
\[ a := 1.541 \quad b := 0.8521 \quad q := 0.012 \quad s := 0.007877 \quad h := -0.00026 \]

\[ P_0 := 31900 \quad P_0 := 50000 \quad P_{\text{max}} := 50000 \]

\[ P(P_0, t, X) := P_0 \cdot \exp\left[-\left(a \cdot (X)^b\right)\right] \cdot \exp\left[q + s \cdot X + h \cdot (X)^2\right] \cdot t \]

\[ i := 0 \ldots 50 \quad j := 0 \ldots 40 \quad X_j := \frac{1.5 \cdot i}{50} \]

\[ T(X, P_{\text{max}}) := \ln\left(\frac{P_{\text{max}} \cdot e^{X \cdot b \cdot a}}{P_0} \right) \cdot \frac{h \cdot X^2 + s \cdot X + q}{40} \]

\[ P := 10000 + (P_{\text{max}} - 10000) \cdot \frac{j}{40} \]

**Figure 4.** The calculation of the dependence of the growth of NMAOAnM from the initial contamination \( P_0, \text{CFU/g} \), shelf life \( t, \text{h} \) and the concentration of HAC in the dressing \( X, \% \) by means of the MathCAD package: a – dependence of NMAOAnM on the initial contamination of freshly prepared salad with dressing without HAC \( P(0) \), shelf life \( t, \text{h} \) and concentration of HAC in the dressing \( X, \% \); b – the shelf life \( t, \text{h} \) from the concentration of HAC in the dressing \( X, \% \) and the initial contamination of freshly prepared salad with dressing without HAC \( P(0) = 50000 \text{ CFU/g} \).

According to this model, the use of the dressing with a HAC at a concentration of 1.0 % during the production of vegetable vinaigrette extends the expiration date to 100 h, which exceeds the expiration date of the vegetable salad with dressing established by SanPiN 2.3.2.1342 (12 h at a temperature \( (4 \pm 2 \) \( \circ \text{C} \)).

This model sets the expiration date at low initial contamination of salads, not exceeding the normative value of NMAOAnM, set CU TR 021/2011 \( (5 \times 10^4 \text{ CFU/g}) \).

When establishing the expiration date of vegetable vinaigrette, ensuring the safety of food products for human health, the model was calculated with an initial contamination at an acceptable level of the normalized NMAOAnM index \( \text{CFU/g} \) in accordance with the requirements of CU TR 021/2011 (figure 5).

**Figure 5.** The effect of the concentration of HAFC in the dressing for the expiration date of vegetable vinaigrette with initial contamination \( P_0 = 5 \times 10^4 \text{ CFU/g} \).

It is established that the expiration date of vegetable salad with an initial dissemination of freshly prepared salad \( P_0 = 5 \times 10^4 \text{ CFU/g} \) and at a HAFC concentration in the 1 % dressing will be 78 h.
The obtained optimal technological mode of production of vegetable vinaigrette with the use of HAC in the dressing according to microbiological indicators is analyzed according to the proposed regression model of the criterion of organoleptic evaluation. To do this, the calculated values of the storage duration (t = 78 h) and the concentration of HAC in the dressing (X = 1 %) are introduced in the formula (4):

\[ K_0 = 0.89844 + 0.208695 \cdot 1 - 0.09714 \cdot 1^2 - 0.00585 + 0.00000103 \cdot 78^2 + 0.002054 \cdot 78 \cdot 1 = 0.72 \]

The obtained value of the organoleptic evaluation criterion is 0.72 and above the permissible level of 0.6. Therefore, the optimal parameter of the technological mode is the concentration of HAC in the dressing of 1 %, extending the expiration date of the vegetable vinaigrette from 12 h to 78 h with the maximum allowable value of the initial contamination (P_0 = 5×10⁴ CFU/g).

The final stage of optimization is the verification of the obtained regression models for microbiological and organoleptic indicators of vegetable vinaigrette during storage.

The results of verification of regression models for microbiological and organoleptic indicators confirm the established shelf life of the vegetable salad with the application of a 1 % solution of HAC in the dressing 78 h.

5. Conclusion

According to the proposed model for optimizing the technological mode of production of chitosan using salads, it was determined that the use of HAFC in the dressing at a concentration of 1% extends the expiration date set by SanPiN 2.3.2.1324 from 12 h to 78 h, ensuring high organoleptic indicators of quality and safety (table 4) during the entire expiration date.

| Table 4. Salads safety indicators after set expiration dates |
|---------------------------------------------------------------|
| Microbiological indicators | Permissible level on CU TR 021/2011 | Value of the indicator |
|------------------------------|-----------------------------|----------------------|
| NMAOAnM, CFU/g               | No more than 5×10⁴         | 0.35×10⁴             |
|                              |                             | 2.70×10⁴             |

The generalization of the experimental results was the basis for the development of salad dressing recipes using HAFC (table 5).

| Table 5. Salad dressing recipe using HAFC |
|-------------------------------------------|
| The name of the recipe components         | Raw material consumption, % |
|-------------------------------------------|-----------------------------|
| Vegetable oil                             | 43.60                       |
| Granulated sugar                          | 3.80                        |
| Ground black pepper                       | 0.20                        |
| Table salt                                | 1.90                        |
| 1 % chitosan solution in 3 % acetic acid  | 0.50                        |
| acid-soluble chitosan                     | 50.00                       |
| 3 % acetic acid                           |                             |

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