CI&CEQ
Chemical Industry & Chemical Engineering Quarterly

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www.ache.org.rs/CICEQ

Chem. Ind. Chem. Eng. Q. 28 (1) 9–17 (2022) CI&CEQ

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SCIENTIFIC PAPER

UDC 637.54.07:664.9:579

STORAGE TIME EFFECT ON INOCULATED, OSMODEHYDRATED CHICKEN MEAT - MICROBIOLOGICAL AND CHEMICAL CHARACTERISTICS

Article Highlights

- Inoculated, osmodehydrated chicken meat was stored at 22 °C, during 14 days
- Microbiological and chemical characteristics were tested and modeled
- During storage, the number of all tested microorganisms on meat samples decreased
- Lipid oxidation occurred after 14 days of storage
- Developed models allows good prediction of tested responses

Abstract

In this research, chicken meat was inoculated with selected microorganisms and subjected to the osmotic dehydration process in two osmotic solutions, in an effort to investigate the effect of storage time duration on its microbiological and chemical characteristics. Total viable counts, numbers of Enterobacteriaceae, Salmonella spp., Listeria monocytogenes, Escherichia coli, proteolytic bacteria, psychotropic bacteria, of microbiological, and biogenic amine content, TBARS and DPPH, and chemical analyses were conducted on meat samples stored at 22 °C, during 14 days. During storage, the number of all tested microorganisms on meat samples decreased. The highest reduction occurred in the first 4 days. The meat dehydrated in molasses achieved better results of microbiological profile during storage. Results of TBARS and DPPH analyses indicated lipid oxidation after 14 days of storage, while the results after 10 days were satisfactory. Developed mathematical models allows good prediction of microbiological and chemical responses of dehydrated chicken meat during the investigated storage duration.

Keywords: chicken meat, E. coli, food safety, Listeria, Salmonella, storage.

In an effort to answer the remarkable recent growth in demand for chicken meat products, producers began to increase the value and shelf life of these products [1].

Osmotic dehydration is a food preservation method, which is important in the food processing industry due to advantages of low processing temperatures, base waste materials and low general energy requirements [2].

Molasses was investigated, in many cases, as an alternative medium for osmotic dehydration. It is characterized by high dry matter (over 80%) and high osmotic pressure, which provide conditions for high reduction of present microorganisms in the raw material that is processed [3].

Water loss, which occurs during the osmotic dehydration process, lowers dehydrating material water activity values, leading to drastically reduced rates of chemical reactions and growth of toxin-producing microorganisms in osmodehydrated materials, consequently providing more stable products during storage [4].

Salmonella is, by far, the most important pathogen derived from poultry that infects human health through food. *E. coli* is an indirect indicator of faecal contamination and especially significant due to the

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Paper received:18 June, 2020
Paper revised: 15 October, 2020
Paper accepted: 22 April, 2021

https://doi.org/10.2298/CICEQ200618011F

highly pathogenic isolate of *E. coli* 0157:H7. *L. monocytogenes*, an important foodborne pathogen, sometimes associated with poultry products, occasionally causing clinical disease in poultry [5].

Biogenic amines in foods occur as products of microorganisms' metabolites, especially microorganisms from groups of *Enterobacteriaceae*, *Enterococcus* and *Lactobacillus* [6].

Lipids in meat and meat products are very easily oxidized, presenting a major cause of meat spoilage in production process, storage and distribution. Products of oxidation, even in low quantities, can reduce storage time and expedite meat spoilage by reducing nutritive values, sensory characteristics decline and compounds toxic to human health accumulate [7,8].

The goal of this research is to analyse and model the effect of storage time duration on the microbiological and chemical characteristics of chicken meat inoculated with selected microorganisms and subjected to the osmotic dehydration process in two osmotic solutions.

EXPERIMENTAL

Osmotic solutions preparation

The aqueous osmotic solution of NaCl and sucrose (aqueous solution) was prepared using comercial sucrose and sodium chloride dissolving 1200 and 350 g to 1 kg of distilled water, respectively [9,10].

"Crvenka" sugar factory, Crvenka, Serbia supplied sugar beet molasses, with initial dry matter content of 85.04%.

Initial microbiological profiles of aqueous soluteions and molasses were analysed according to standard ISO methods for selected microorganisms. The results were: <10 colony forming unit per g (cfu/g) for *E. coli* and *Enterobacteriaceae*; negative in 25 g for *Salmonella* spp. and *L. monocytogenes*. Standard ISO methods limit of quantification (*LOQ*) for enumeration of tested microorganisms was: <10 cfu/g, while the limit of detection (*LOD*) for *Salmonella* spp. and *L. monocytogenes* was <1 cfu in 25 g.

Chicken breast meat preparation

The raw skinless chicken breast meat was obtained just prior to the experiments, at the local market in Novi Sad, Serbia. Cube samples, dimensions of: 1 cm×1 cm×1 cm, were cut from whole lean muscle, by a sterilized knife with a stainless-steel blade on a sterilized plastic board [11,12].

The microbiological profile of raw meat samples was determined in accordance with the European Commission Regulation No 2073/2005 [13], where meat samples were divided into five sub-samples and individually tested according to standard ISO methods for each selected microorganism. The results were: <10 cfu/g for *E. coli* and *Enterobacteriaceae;* negative in 25 g for *Salmonella* spp. and *L. monocytogenes. LOQ* was the same as mentioned in the previous section, for the osmotic solutions preparation.

Inoculation and incubation of chicken meat samples

In this research, *Salmonella typhimurium* ATCC 14028, *Salmonella enteritidis* ATCC 13076, *E. coli* ATCC 25922, E. *coli* ATCC 8739, *L. monocytogenes* ATCC 19111 and *L. monocytogenes* ATCC13932 (Microbiologics, St. Cloud, MN, USA) were used as reference strains. Commercial reference cultures were stored and maintained in accordance to ISO 11133:2014 [14], and the procedures of activation, preparation, refreshment of working culture and preparation of initial suspension of selected microorganisms were described in detail in Filipović *et al.* [15].

Chicken meat samples were inoculated with 0,1 ml of the 10^{-2} dilution of a cocktail mixture containing the three-strains of test microorganisms. Approximately 50,000 cfu/ml or of each test microorganism, or 150,000 cfu/g of chicken meat was achieved. Inoculated chicken meat samples were stored at room temperature for a duration of 60 min, to allow the successful attachment of the test microorganisms on the surface of chicken meat samples.

After 1 h storage, chicken meat cubes were sampled and prepared for the analysis at the beginning of the process marked as: 0 hr [12].

Osmotic dehydration process

The process of osmotic dehydration of inoculated chicken meat samples was the same as explained in detail in Filipović *et al.* [12], where the most important process parameters were process temperature of 32 °C, under atmospheric pressure and duration of the process of 5 h. Part of the inoculated osmodehydrated chicken meat samples in triplicates were subjected to the microbiological and chemical analyses, after the osmotic dehydration process, and before storage.

Methods of microbiological analysis

Counting of the total viable count (TVC) was done according to the standard method ISO 4833-1:2014 [16].

Identification of *Enterobacteriaceae* was performed in accordance to the standard method ISO 21528-2:2017 [17].

Enumeration of *Salmonella* spp. was performed according to the modified standard method ISO 6579-

-1:2017 [18]. Modification of the standard method is described in detail in Filipović *et al.* [15].

Detection of *Salmonella* spp. was performed according to the standard method ISO 6579-1:2017 [18].

Enumeration and detection of *L. monocytogenes* were performed in accordance with the standard methods ISO 11290-2:2017 [19] and ISO 11290--1:2017 [20], respectively.

Determination of the number of *E. coli* was performed in accordance with the standard method ISO 16649-2:2001 [21].

Identification of proteolytic bacteria was performed in accordance with the experimental procedure explained in Němečková *et al.* [22].

Enumeration of psychotropic bacteria was performed in accordance with standard method NMKL Method 86, 5th Ed. [23].

Methods of chemical analysis

Biogenic amines content determination was performed in accordance with the experimental procedure explained in Duflos *et al.* [24].

2-thiobarbituric acid reactive substances (TBARS) test was performed in accordance with the experimental method of Botsoglou *et al.* [25], with modifications described by Šojić *et al.* [26]. TBARS values were expressed as milligrams of malondialdehyde per kilogram of the sample (mg MDA/kg).

The antioxidative activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity assay in accordance with the experimental procedure explained in Vaštag *et al.* [27]. For the purpose of presenting the samples' antioxidative activity, the value of 50% of maximum inhibitory concentration (IC_{50}) is used, calculated as the concentration of samples in μg/ml, which is required for the reduction of 50% of antioxidative activity, under applied conditions [28].

Packaging and storage

50 g of inoculated, osmotically dehydrated chicken meat samples were packed in sterile "Whirl-Pak" sampling bags (Nasco, USA), which were closed by a VS 110W device (Gorenje, Slovenija), for the samples' moisture change prevention. Packed meat samples were stored in a thermostat (ST 1 Basic, Pol-Eko, Poland), at the storage temperature of 22 °C for time periods of: 1, 2, 4, 7, 10 and 14 days.

After every storage period, meat samples in triplicates were subjected to the same microbiological and chemical analyses as in the case of meat samples tested after the osmotic dehydration process, and before storage.

After 14 days of storage, the analysis of detection of *Salmonella* spp. and *L. monocytogenes* in 10 g of meat samples was performed.

Methods of statistical analysis

Based on experimental data for microbiological and chemical analysis of the inoculated osmodehydrated chicken meat, mathematical models of dependence of microbiological and chemical responses from storage time, as an independent variable, are formed:

$$Y_k = f_k(storage\_time) \tag{1}$$

Third order polynomial is used for the approximation of experimental data. Models of 8 responses (TVC, *Enterobacteriaceae*, *Salmonella* spp., *L. monocytogenes*, *E. coli*, psychotropic bacteria, TBARS and DPPH) in dependence of 1 independent variable (storage time) are developed:

$$Y_k = a_{k0} + a_{k1}X + a_{k2}X^2 + a_{k3}X^3, k = 1-8 \tag{2}$$

where: $a_{k0\text{-}3}$ are regression coefficients; Y are TVC (Y_1), *Enterobacteriaceae* (Y_2), *Salmonella* spp. (Y_3), *L. monocytogenes* (Y_4), E. *coli* (Y_5), psychotropic bacteria (Y_6), TBARS (Y_7) and DPPH (Y_8), while X is storage time.

For the microbiological and chemical responses of the inoculated osmodehydrated chicken meat analysis, nonlinear least square regression analysis with the Levenberg-Marquardt method of estimation is used.

The quality of developed models was tested using coefficient of determination (R^2), reduced chi-square (χ^2), mean bias error (*MBE*), root mean square error (*RMSE*) and mean percentage error (*MPE*), calculated as explained in Arsenović *et al.* [29].

The significance of the effect and interaction of individual factors, for every response was determined by analysis of variance (ANOVA) and application of post-hoc Tukey's HSD test.

For the purpose of reliable ANOVA testing, *LOQ* values below 10 cfu/g were substituted with values *LOQ*/2 or 5 cfu/g [30].

For ANOVA and third order polynomial models calculation, StatSoft Statistica ver.12.0. software package was used, and for calculation of the quality of the developed models' parameters, Microsoft Excel ver. 2016 was used.

RESULTS AND DISCUSSION

Table 1 shows average values and standard deviations of microbiological responses of inoculated, osmotically dehydrated chicken meat in two osmotic

*Table 1. Average values and standard deviations of microbiological responses of inoculated, osmotically dehydrated chicken meat during storage; [a-f] - different letters in the superscript between values in the same column, for both osmotic solutions, indicate statistically significant difference, at the level of significance of p < 0.05 (based on post-hoc Tukey's HSD test); * - values (0.05±0.00)×10² present LOQ/2, used for the purpose of ANOVA testing, where substituted, measured data were <10 cfu/g*

Solution	Storage time (days)	Microorganism (cfu/g)						
		TVC (×10²)	*Enterobacteriaceae* (×10²)	*Salmonella* spp. (×10²)	*L. monocytogenes* (×10²)	*E. coli* (×10²)	Proteolytic bacteria (×10²)	Psychotropic bacteria (×10²)
Aqueous solution	0	165.00±7.07^{f}	155.00±21.21^{e}	120.00±28.28^{e}	5.20±0.85^{f}	19.00±2.83^{f}	<0.10	9.75±1.77^{f}
	1	80.00±4.24^{d}	71.50±10.60^{c}	55.50±4.95^{cd}	3.75±0.63^{e}	7.45±0.92^{de}	<0.10	4.45±1.20^{de}
	2	80.50±9.19^{d}	72.50±7.78^{c}	56.50±12.02^{bc}	2.10±0.99^{cd}	7.50±0.99^{cd}	<0.10	4.45±0.50^{cd}
	4	18.50±3.54^{b}	15.00±2.83^{a}	11.80±3.11^{a}	1.25±0.78^{bc}	2.05±0.78^{ab}	<0.10	2.85±0.49^{bc}
	7	3.00±0.85^{ab}	2.00±0.28^{a}	1.15±0.35^{a}	0.70±0.14^{ab}	0.40±0.00^{a}	<0.10	1.70±0.28^{ab}
	10	2.00±0.14^{ab}	0.05±0.00^{a} *	0.05±0.00^{a}	0.50±0.14^{ab}	0.05±0.00^{a}	<0.10	1.55±0.35^{ab}
	14	1.15±0.35^{a}	0.05±0.00^{a}	0.05±0.00^{a}	0.05±0.00^{a}	0.05±0.00^{a}	<0.10	0.05±0.00^{a}
Molasses solution	0	145.00±7.07^{e}	130.00±14.14^{d}	109.00±15.56^{e}	2.80±0.42^{de}	11.85±3.04^{e}	<0.10	7.45±0.49^{e}
	1	94.50±4.95^{d}	91.00±5.66^{c}	82.00±8.49^{d}	1.45±0.49^{bc}	7.35±0.77^{cd}	<0.10	4.25±0.77^{cd}
	2	60.00±5.66^{c}	49.00±5.66^{b}	40.50±4.95^{b}	1.25±0.49^{bc}	4.75±0.64^{bc}	<0.10	2.85±0.35^{bc}
	4	9.55±1.48^{ab}	8.00±0.85^{a}	5.95±0.92^{a}	1.05±0.35^{a-c}	1.00±0.14^{a}	<0.10	1.50±0.42^{ab}
	7	1.50±0.42^{ab}	0.75±0.21^{a}	0.60±0.14^{a}	0.50±0.00^{ab}	0.05±0.00^{a}	<0.10	0.55±0.21^{a}
	10	1.00±0.42^{a}	0.05±0.00^{a}	0.05±0.00^{a}	0.05±0.00^{a}	0.05±0.00^{a}	<0.10	0.05±0.00^{a}
	14	0.75±0.21^{a}	0.05±0.00^{a}	0.05±0.00^{a}	0.05±0.00^{a}	0.05±0.00^{a}	<0.10	0.05±0.00^{a}

solutions (aqueous solution and molasses) during storage (from 0 to 14 days) at 22 °C.

Analysing levels of selected microorganisms' survival and the possibility to grow and multiply on the inoculated, osmotically dehydrated chicken meat during storage, would allow the possibility to define osmotically dehydrated chicken meat shelf life during which it is safe to use.

Chicken meat subjected to the osmotic dehydration process, in duration of 5 h at 32 °C, is characterized by: 57.11 and 64.76% of dry matter content, 0.4702 and 0.5283 g of water loss per g of initial sample, 0.1336 and 0.1443 g of solid gain per g of initial sample, and a_w values of 0.835 and 0.812 for aqueous solution and molasses, respectively [11,31].

TVC values, just after the osmotic dehydration process and before the beginning of the storage phase (time point marked as: 0 days), indicate that the type of osmotic solution statistically significantly affected levels of survived total bacteria. TVC levels were lower in inoculated chicken meat dehydrated in molasses, which is in accordance with previous researches [12,14].

With storage time, a statistically significant decrease of TVC values occurred. TVC values in every storage time point were lower for the inoculated chicken meat dehydrated in molasses in comparison to meat dehydrated in aqueous solution. After 14 days of storage, TVC values were reduced to 1.15×10^2 and 0.75×10^2 cfu/g for meat samples dehydrated in aqueous solution and molasses, respectively. This high reduction of TVC values during storage can be explained by bacteria cell plasmolysis phenomena which occurs during prolonged exposure of the cells to the high osmotic pressure environment, inducing bacterial cell damage [32]. Combined with the low achieved osmotically dehydrated chicken meat a_w values [11], which were lower from limiting a_w values for growth and multiplication of most microorganisms [33,34], initially present microorganisms eventually die out. Statistically significant reduction of *TVC* values of inoculated, osmotically dehydrated chicken meat in aqueous solution occurred during 7 days of storage, while in the case of molasses as an osmotic solution, it occurred only during 4 days of storage. After these periods of storage, reductions of numbers of present microorganisms on chicken meat were not statistically significant, since the levels of present microorganisms at these storage time points were low.

After the osmotic dehydration process and before the storage, the numbers of *Enterobacteriaceae* were statistically significantly different depending upon applied osmotic solutions. Higher numbers of *Enterobacteriaceae* were determined in meat dehydrated in aqueous solution, as in the case of *TVC*. During the storage time, the same as of *TVC*, numbers of *Enterobacteriaceae* on meat samples were statistically significantly reduced.

In the first days of storage (up to 7 days of storage), the number of *Enterobacteriaceae* was

lower on meat samples dehydrated in molasses in comparison to the meat samples dehydrated in aqueous solution. After 4 days of storage there was no statistically significant reduction, while after 10 days of storage, numbers of *Enterobacteriaceae* were <10 cfu/g for all meat samples. Reduction of numbers of *Enterobacteriaceae* can be explained, as in the case of *TVC* and as in cases of all following microorganisms, by achieving low a_w values of chicken meat in dehydration processes [11]. This led to total reduction of initial numbers of *Enterobacteriaceae.*

Statistically significant differences between *E. coli* values after osmodehyration process and before storage, in dependence of applied osmotic solutions, were noted. The same as in cases of previous microorganisms, lower values of *E. coli* were achieved on meat samples, dehydrated in molasses, at every time point. Storage time statistically significantly reduced values of *E. coli* on meat samples, dehydrated in both solutions. After 4 days of storage of meat samples dehydrated in both solutions, there was no more statistically significant reduction of *E. coli* values. Final *E. coli* value (<10 cfu/g) was achieved after 7 and 10 days of storage of meat dehydrated in molasses and aqueous solution, respectively.

The number of *Salmonella* spp. on inoculated chicken meat after the process of osmotic dehydration and before storage, was not statistically significantly different depending upon the type of osmotic solution, but it was still lower on meat samples dehydrated in molasses.

Statistically significant reduction of numbers of *Salmonella* spp. occurred during storage time of inoculated, osmotically dehydrated chicken meat, in both osmotic solutions, as in the cases of previously tested microorganisms. The number of *Salmonella* spp. was statistically insignificantly lower on meat samples dehydrated in molasses than in aqueous solution at corresponding storage time points. As in the case of *Enterobacteriaceae*, just after 4 days of storage there was no further statistically significant reduction of the number of *Salmonella* spp., while after 10 days of storage, numbers of *Salmonella* spp. were <10 cfu/g for meat samples dehydrated in both solutions.

By the *Salmonella* spp. detection analysis, in 10 g of inoculated, osmotically dehydrated chicken meat in both osmotic solutions, after 14 days of storage, *Salmonella* spp. was not detected.

Number of *L. monocytogenes* on inoculated chicken meat, after the osmotic dehydration process in both solutions, before storage, was significantly lower in comparison to other tested microorganisms. Number of *L. monocytogenes* on meat samples was statistically significantly different in dependence of applied osmotic solutions, where the process with molasses gained better results in terms of higher reduction of present *L. monocytogenes*.

With storage time, statistically significant reduction of numbers of *L. monocytogenes* on meat dehydrated in both solutions occurred. After 7 days of storage, further reduction of numbers of *L. monocytogenes* on meat osmodehydrated in aqueous solution was not statistically significant, while after 14 days of storage it reached the final value of <10 cfu/g. In the case of molasses, already after 4 days of storage the further reduction of numbers of *L. monocytogenes* was not statistically significant, and after 10 days of storage reached the final value of <10 cfu/g.

By the *L. monocytogenes* detection analysis, in 10 g of meat, in both osmotic solutions, after 14 days of storage, *L. monocytogenes* was not detected.

Results of proteolytic bacteria analysis on the inoculated, dehydrated chicken meat, in both osmotic solutions, showed that in all tested samples the results were <10 cfu/g.

In dependence of applied osmotic solutions, the number of psychotropic bacteria was statistically significantly different, only at time point of 0 days of storage, while during the storage, there was no statistically significant difference. As in the cases of previously tested microorganisms, lower numbers of psychotropic bacteria were obtained using molasses in the process. With storage time, the statistically significant reduction of the number of psychotropic bacteria occurred. After 7 days of storage of inoculated chicken meat dehydrated in aqueous solution, there was no more statistically significant reduction in the number of psychotropic bacteria, while after 14 days of storage, the value of <10 cfu/g was achieved. In the case of meat dehydrated in molasses, only after 4 days of storage, there was no more statistically significant reduction in the number of psychotropic bacteria, while after 10 days of storage, the value indicating total reduction of <10 cfu/g was achieved.

Table 2 shows the results of chemical analysis of inoculated chicken meat osmodehydrated in two different solutions, during the storage time frame of 0 to 14 days, at the temperature of 22 °C.

Results of biogenic amines content of meat, dehydrated in both osmotic solutions, have showed that in all tested samples, at all storage time points, values were less than 10 mg/kg. These data are in accordance with the results of the number of proteolytic bacteria on inoculated, osmodehydrated chicken meat, which stayed unchanged during the tested storage time frame (<10 cfu/g), since biogenic amines

are products of proteolytic bacteria metabolism [35]. The results of meat samples' biogenic amines content also indicate that, although individual proteolytic bacteria (*E. coli* and *L. monocytogenes*) testing showed their presence, there was no metabolic activity present during storage time frame.

Table 2. Average values and standard deviations of chemical responses of inoculated, osmotically dehydrated chicken meat during storage; biogenic amines: <10 mg/kg; [a-f] - different letters in the superscript between values in the same columns, indicate statistically significant difference, at the level of significance of p < 0.05 (based on post-hoc Tukey's HSD test)

Solution	Storage time (days)	*TBARS* (mg/kg)	DPPH IC_{50} (mg/ml)
Aqueous solution	0	0.17±0.01[a]	27.38±0.15[bc]
	1	0.19±0.01[ab]	29.80±0.06[d]
	2	0.22±0.01[bc]	31.16±0.31[ef]
	4	0.23±0.01[c]	31.50±0.27[f]
	7	0.28±0.01[e]	31.45±0.58[f]
	10	0.36±001[g]	32.11±0.08[f]
	14	0.51±0.02[i]	38.37±1.38[g]
Molasses solution	0	0.21±0.02[bc]	25.57±0.01[a]
	1	0.24±0.01[cd]	26.87±0.10[ab]
	2	0.26±0.01[d]	27.34±0.02[bc]
	4	0.29±0.01[e]	28.64±0.01[cd]
	7	0.33±0.01[f]	29.22±0.10[d]
	10	0.39±0.01[h]	29.90±0.04[de]
	14	0.60±0.01[j]	31.60±0.48[f]

Based on the results of *TBARS*, it can be seen that there is a statistically significant difference between meat samples dehydrated in different solutions. The results of different *TBARS* values in dependance of used osmotic solution, can be explained by different colours of osmodehydrated samples when aqueos solution and molasses are used [36]. *TBARS* values determination method is a spectrophotometric one [25], which the colour of the samples affects. From these reasons, direct comparison of *TBARS* results between samples subjected to different processes, can produce contradictory conclusions.

With the meat storage time, a statistically significant increase of *TBARS* values occured. After 14 days of storage, *TBARS* values of meat samples dehydrated in both solutions surpassed value of 0.5 mg/kg, which is considered a limit for fat rancidity [26]. However, meat samples, after 10 days of storage, were still characterized with low *TBARS* values (0.36 and 0.39 mg/kg for samples dehydrated in aqueous solution and molasses, respectively). The explanation of the prolonged storage period at temperature of 22 °C before reaching fat rancidity limit can be the same as in the case of the reduction of number of tested microorganisms during storage time. Unfavorable conditions for growth and metabolic activity of most microorganisms probably affect lipolitic microorganisms as well (which were not investigated in this research).

Values of DPPH, expressed as IC_{50} values, show that statistically significantly lower IC_{50} values, or higher antioxidative activity, are noted in meat dehydrated in molasses rather than in aqueous solution, at all storage time points. These obtained results are in accordance with literature data of Nićetin *et al.* [37] and Knežević *et al.* [38], where it is explained that in the osmodehydration process, when molasses is used as osmotic solution, the increase of dehydrated raw material antioxidative activity occurs. During the osmodehydration process, molasses via secondary mass transfer is incorporated in dehydrating raw material solid matter [3]. Considering that molasses contains numerous antioxidative compounds [39], the antioxidative activity of dehydrating raw material is also increased.

With storage time, values of DPPH IC_{50} increased, or antioxidative activity of inoculated chicken meat, dehydrated in both solutions, decreased. These results are in correlation with *TBARS* results of meat samples during storage, indicating lipid oxidation and free radicals formation, which decrease antioxidative activity and reduce nutritive and sensory characteristics of food during storage [40]. Statistically significant increase of DPPH IC_{50} values, or decrease of antioxidative activity, occurred after 14 days of storage of inoculated chicken meat samples, osmodehydrated in both osmotic solutions.

Supplementary tables available from the author upon request show regression coefficients and fit quality parameters of developed mathematical models of dependence of microbiological and chemical responses of inoculated, osmotically dehydrated chicken meat from the time of the storage, in the form of third order polynomial, Eq. (2).

Based on third order polynomial Eq. (2) regression coefficients (a_0-a_3), shown in Supplementary Tables, mathematical models of microbiological and chemical responses dependence from the time of the storage of inoculated, osmotically dehydrated chicken meat in aqueous solution and molasses, respectively, can be formed. The slopes of model curves (coefficient a_1) were negative for microbiological parameters, for both osmotic solutions, statistically significant at $p < 0.05$ level, which demonstrate the decreasing trend of the observed microbiological parameters during storage time. The opposite trend was

noticed for the chemical parameters, where the positive sign slope indicated the increase in *TBARS* and DPPH during the storage time.

Statistically significant were linear coefficients for all tested responses in both applied osmotic solutions, except for *L. monocytogenes* for chicken meat dehydrated in molasses. In cases of *L. monocytogenes* and DPPH for chicken meat dehydrated in aqueous solution and *TVC*, *Enterobacteriaceae*, *Salmonella* spp., *E. coli*, TBARS and DPPH for chicken meat dehydrated in molasses, quadratic and cubic coefficients were also statistically significant.

From the comparison of the coefficients' statistical significance from mathematical models for aqueous solution and molasses, more profound effects of storage time on inoculated, osmodehydrated chicken meat in molasses microbiological and chemical responses can be seen.

A mathematical model for proteolytic bacteria is not developed, since the obtained experimental data did not change with the change of the independent variable (storage time). Supplementary Tables also show quality fit parameters of the developed mathematical models from which can be seen that all developed models were characterized by high R^2 values and low χ^2, *MBE*, *RMSE* and *MPE* values, indicating correspondence of calculated values with experimental data [29].

Based on developed TBARS mathematical models for inoculated, osmotically dehydrated chicken meat in aqueous solution and molasses, storage times with assigned *TBARS* values of 0.5 mg/kg (fat rancidity limiting value [26]) were calculated. The storage times of inoculated, osmotically dehydrated chicken meat in aqueous solution and molasses, in the moment of rancidity occurrence, were 13.75 and 12.48, respectively.

CONCLUSIONS

Inoculated chicken meat samples dehydrated in molasses were characterized by lower values of all tested microorganisms (*TVC*, *Salmonella* spp., *L. monocytogenes*, *E. coli*, *Enterobacteriaceae* and psychotropic bacteria) at all storage time points in comparison to the samples dehydrated in aqueous solution. With storage time, the number of all tested microorganisms on all meat samples decreased and the highest reduction occurred in the first 4 days of storage. Numbers of all tested microorganisms, except *TVC*, after 14 days and in some cases only after 7 days of storage, were <10 cfu/g.

The conditions for survivability on inoculated chicken meat dehydrated in both solutions were the least favourable for *L. monocytogenes* in comparison to other microorganisms.

Biogenic amines content showed that there was no metabolic activity of proteolytic bacteria and no protein degradation during meat storage.

Results of the *TBARS* and DPPH indicate that lipid oxidation occurred after 14 days of storage, while meat samples stored during 10 days were characterized by satisfactory levels of these responses.

All developed mathematical models of microbiological and chemical responses of inoculated, osmodehydrated chicken meat in dependence of storage time showed good correlation between calculated and experimental values and allow good prediction of tested responses.

Supplementary material

Additional data are available from the corresponding author upon request.

Acknowledgment

The presented data is the result of investigation financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number: 451-03-68/2020-14/ 200134.

REFERENCES

[1] G. Volpato, E.M.Z. Michielin, S.R.S. Ferreira, J.C.C. Petrus, J. Food. Eng. 79 (2007) 779-785

[2] A.A. El-Aouar, M.P. Azoubel, L.J. Barbosa Jr., F.E.X. Murr, J. Food. Eng. 75 (2006) 267-274

[3] V. Filipović, B. Ćurčić, M. Nićetin, D. Plavšić, G. Koprivica, N. Mišljenović, Hem. Ind. 66 (2012) 743-748

[4] C. Tortoe, Afr. J. Food. Sci.4 (2010) 303-324

[5] B.M. Hargis, D.J. Caldwell, J.A. Byrd, in Poultry Meat Processing, C.M. Owens, C.Z. Alavarado, A.R. Sams Ed.(s), CRC Press Taylor & Francis Group, Boca Raton, FL, 2010, p. 121

[6] P. Dalgaard, J. Emborg, in Foodborne pathogens – Hazards, risk analysis and control, C. de Blackburn, P. McClure Ed.(s), 2nd ed., Woodhead Publishing Ltd., Cambridge, 2009, p. 294

[7] L. Zhao, Y. Jin, C. Ma, H. Song, H. Li, Z. Wang, S. Xiao, Meat. Sci. 88 (2011) 761-766

[8] D. Ansorena, I. Astiasarán, Meat. Sci. 67 (2004) 237-244

[9] A. Collignan, P. Bohuon, F. Deumier, I. Poligne, J. Food. Eng. 49 (2001) 153-162

[10] H. Qi, M. Le Maguer, S.K. Sharma, J. Food. Process. Eng. 21 (1998) 75-88

[11] I. Filipović, B. Ćurčić, V. Filipović, M. Nićetin, J. Filipović, V. Knežević,. Food. Process. Pres. 41 (2017)

[12] I. Filipović, S. Markov, V. Filipović, J. Filipović, V. Vujačić, L. Pezo, J. Food. Process. Pres. 43 (2019) e14144

[13] Commission Regulation (EC), No. 2073/2005: On microbiological criteria for foodstuffs, 2005

[14] EN ISO 11133: Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media, 2014

[15] I. Filipović, S. Markov, V. Filipović, J. Filipović, A. Vidaković, N. Novković, V. Rafajlovska, J. Appl. Microbiol. 125 (2018) 843-852

[16] EN ISO 4833-1: Microbiology of the food chain - Horizontal method for the enumeration of microorganisms Colony count at 30 degrees C by the pour plate technique, 2014

[17] EN ISO 21528-2: Microbiology of the food chain - Horizontal method for the detection and enumeration of *Enterobacteriaceae* -- Part 2: Colony-count technique, 2017

[18] EN ISO 6579-1: Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Horizontal method for the detection of Salmonella spp (2017).

[19] EN ISO 11290-2: Microbiology of the food chain – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. – Part 2: Enumeration method, 2017

[20] EN ISO 11290-1: Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria* monocytogenes and of *Listeria* spp. - Part 1: Detection method, 2017

[21] EN ISO 16649-2: Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of beta-glucuronidase-positive *Esherichia*. coli – Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide, 2001

[22] I. Němečková, M. Pechačová, P. Roubal, Czech. J. Food. Sci. 27 (2009) 82-83

[23] NMKL Method 86, 5th ed., Aerobic Microorganisms. Determination in foods at 37°C, 30°C, 25°C, 20°C, 17/7°C or 6.5°C by the colony count method, 2013

[24] G. Duflos, C. Dervin, P. Malle, S. Bouquelet, J. AOAC. Int. 82 (1999) 1097-1101

[25] N.A. Botsoglou, D.J. Fletouris, G.E. Papageorgiou, V.N. Vassilopoulos, A.J. Mantis, A.G. Trakatellis, J. Agric. Food. Chem. 42 (1994) 1931-1937

[26] B. Šojić, V. Tomović, S. Kocić-Tanackov, S. Škaljac, P. Ikonić, N. Džinić, N. Živković, M. Jokanović, T. Tasić, S. Kravić, Food. Control. 54 (2015) 282-286

[27] Ž. Vaštag, S. Popović, Lj. Popović, V. Krimer, D. Peričin, Food. Bioprod. Process. 88 (2010) 277-282

[28] B. Šojić, V. Tomović, M. Jokanović, P. Ikonić, N. Džinić, S. Kocić-Tanackov, Lj. Popović, T. Tasić, J. Savanović, N. Živković Šojić, Czech. J. Food. Sci. 35 (2017) 189-193

[29] M. Arsenović, L. Pezo, S. Stanković, Z. Radojević, Appl. Clay. Sci. 115 (2015) 108-114

[30] J. Kurlj, J. Đisalov, A. Bočarov-Stančić, L. Pezo, J. Kojić, A. Vidaković, M. Bodroža, World. Mycotoxin. J. 11 (2018) 247-257

[31] V. Filipović, B. Lončar, M. Nićetin, V. Knežević, D. Šuput, T. Kuljanin, in Proceedings of II Intenational Congress "Food Technology, Quality and Safety", Novi Sad, Serbia (2014), pp. 94-99

[32] G. Tortora, B. Funke, C. Case, Microbiology an introduction, 11th ed., Pearson Education, Inc, Glenview, 2013, p. 153

[33] T. Huang, W. Nip, in Meat Science and Application, Y. H. Hu, W.K Nip, R.W. Rogers, O.A. Young, Ed.(s), CRC Press Taylor & Francis Group, Boca Raton, FL, 2001, p. 408

[34] G. Feiner, Meat products handbook: Practical science and technology. Woodhead Publishing Ltd, Cambridge, 2006, p. 629

[35] R. Mendes, in Fishery products Quality, Safety and authenticity, H. Rehbein, J. Oehllenschlager Ed.(s), Blackwell Publishing Ltd, Oxford, 2009, p. 42

[36] V. Knežević, B. Ćurčić, V. Filipović, M. Nićetin, Lj. Lević, T. Kuljanin, J. Gubić, J. Process. Energy Agric. 17 (2013) 39-42

[37] M. Nićetin, L. Pezo, B. Lončar, V. Filipović, D. Šuput, V. Knežević, J. Filipović, J. Serb. Chem. Soc. 82 (2017) 253-265

[38] V. Knežević, L. Pezo, B. Lončar, V. Filipović, M. Nićetin, S. Gorjanović, D. Šuput, Period. Polytech. Chem. Eng. 63 (2019) 491-498

[39] V. Valli, A. Gomez-Caravaca, M. Di Nunzio, F. Danesi, M. Fiorenza Caboni, A. Bordoni, J. Agric. Food. Chem. 60 (2012) 12508-12515

[40] J. Yeo, M.K. Jeong, J. Lee, Food. Sci. Biotechnol. 21 (2012) 199-203.

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NAUČNI RAD

UTICAJ VREMENA SKLADIŠTENJA NA MIKROBIOLOŠKE I HEMISJKE KARAKTERISTIKE INOKULISANOG I OSMOTSKI DEHIDRISANOG PILEĆEG MESA

U ovom istraživanju pileće meso je inokulisano sa odabranim mikroorganizmima i podvrgnuto procesu osmotske dehidrtacije u dva osmotska rastvora, sa ciljem ispitivanja uticaja dužine vremena skladištenja na mikrobiološke i hemijske karakeristike pilećeg mesa. Na uzorcima mesa koji su skladišteni tokom 14 dana na temperaturi od 22 °C izvedene su sledeće mikrobiološke analize: ukupan broj bakterija, broj Enterobacteriaceae, Salmonella spp., Listeria monocytogenes, Escherichia coli, proteolitičkih i psihrotrofnih bakterija i sledeće hemijske analize: sadržaj biohenih amina, TBARS i DPPH. Tokom skladištenja, broj svih ispitivanih mikroorganizama na uzorcima mesa se smanjio. Najveće smanjenje se javilo tokom prva četiri dana. Meso dehidrirano u melasi je pokazalo bolje rezultate mikrobiološkog profila tokom skladištenja. Rezultati TBARS i DPPH analiza su ukazali na pojavu oksidacije lipida nakon 14 dana skladištenja, dok su rezultati nakon 10 dana skladištenja bili zadovoljavajući. Razvijeni matematički modeli su pokazali dobro predviđanje mikorbioloških i hemijskih odziva dehidriranog mesa tokom ispitivanog vremena skladištenja.

Ključne reči: pileće meso, E. coli, zdravstvena bezbednost hrane, Listeria, Salmonella, skladištenje.