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Changes in total viable count and TVB-N content in marinated chicken breast fillets during storage

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Abstract. Marination is a popular technique for enhancing meat properties. Depending on the marinade type and ingredients added, marination can improve sensory, chemical and microbiological quality of meat products. In this study, the total viable count and total volatile basic nitrogen (TVB-N) content in marinated chicken breast fillets were investigated. The possible correlation between bacterial growth and formation of TVB-N was also tested. Chicken breast fillets were immersed in a solution of table salt (as a control) or three different marinades, which consisted of table salt, sodium tripolyphosphate and/or sodium citrate, and stored in air for nine days at 4±1°C. Analyses of the total viable count and TVB-N were performed on days 0, 3, 6 and 9 day of storage. The total viable count gradually increased in all examined groups, and statistically significant differences ($p < 0.01$; $p < 0.05$) between treatments on days 0, 3 and 6 day of storage were established. TVB-N values in marinated chicken were significantly higher ($p < 0.01$; $p < 0.05$) compared to the control. Using the multiple linear regression, a positive correlation between total viable count and formation of TVB-N in chicken marinated with sodium citrate was established ($p < 0.05$), while the intensity of TVB-N formation was lowest in chicken marinated with sodium tripolyphosphate.

1. Introduction

Poultry meat has become a mass consumer product throughout the world: in every region, in countries with very different levels of development and in diverse forms. Furthermore, as an important source of proteins, poultry meat has a high biological value and it has been frequently recommended for its nutritious low-fat content [1]. In the last few decades, consumers' demand for convenience foods has resulted in an expansion of the processed meat and poultry industry [2].

The food industry constantly seeks new ways to add value to products, and one example is the marination of raw meat to add flavour and extend shelf-life [3]. Marination also improves the tenderness, juiciness, flavour, colour, and cooking yield of meat and poultry [4]. Depending on marinade type, marination might affect chemical and sensory attributes of the meat, as well as the microbiological safety of the final meat product. For the marination of poultry meat, alkaline marinades are often used. A typical marinade solution for commercial chicken products is made of 90% water, 6% table salt (sodium chloride), and 4% sodium tripolyphosphate (STPP) [5]. However, the quantity of phosphate used in meat products is limited by legislation [6], and more often the



phosphates are combined with other additives in order to increase the water binding capacity of the meat. Sodium citrate has been utilized as a phosphate replacer to enhance water-holding properties of the meat [7]. Although these additives are not considered as antimicrobials, studies of the antimicrobial activity of sodium tripolyphosphate and other polyphosphates on food contaminants have been conducted suggesting their possible use as antimicrobials [8-10]. According to standard NF-V01-003 [11], total viable counts in marinated chicken meat should not exceed the maximum recommended limit of 5.7 log CFU/g of meat.

In addition to microbial counts, some chemical parameters could indicate meat freshness. The total volatile basic nitrogen (TVB-N) content is a chemical indicator of the meat quality, and it is associated with the amino acid decarboxylase activity of microorganisms during storage. The above-mentioned standard [11] recommends that TVB-N value in marinated chicken meat should not exceed 60 mgN/100g of the meat, while Balamatsia *et al.* [12] suggested a value of 40 mg N/100 g as an upper limit for fresh poultry meat.

This study was conducted to investigate the changes in a total viable count and TVB-N content in marinated chicken breast fillets during storage. Therefore, the addition of STPP and sodium citrate to chicken breast fillets as a means of enhancing sensory quality, and the effect of various concentrations of STPP (1 and 2%) and sodium citrate (1 and 2%), on the behaviour of total viable count and formation of TVB-N on days 0, 3, 6 and 9 of storage at 4.0±1°C were investigated. The possible correlation between bacterial growth and formation of TVB-N was also tested.

2. Materials and Methods

Deboned and skinless chicken breast meat (*m. pectoralis major*) was purchased in a local poultry abattoir. In the laboratory, meat was aseptically filleted into pieces weighing approximately 0.1 kg and divided into four groups.

For marinade, table salt with 99 to 99.5% sodium chloride (Solana dd Tuzla, Bosnia and Herzegovina), a “Carfosel Genius” commercial mixture of sodium tripolyphosphate (Prayon, Belgium), tri-sodium citrate dihydrate ($C_6H_5Na_3O_7 \times 2H_2O$) (Merck, Germany) and drinking water were used. Three different marinades and a solution of 6% table salt as a control (group I) were prepared and kept refrigerated 24 hours at 4±1°C before use. The first marinade consisted of water, 6% NaCl and 2% phosphate (group II), the second consisted of water, 6% NaCl and 2% citrate (group III) and the third one consisted of water, 6% NaCl, 1% phosphate and 1% citrate (group IV). Chicken breast fillets were immersed in marinade solutions or in the control solution of table salt in the ratio of 1:2 (100g of meat/200 mL of marinade or table salt solution). Marination process lasted 4h at 4±1°C, with stirring at times. After 4 h of immersing, the fillets were drained, packed in sterile Stomacher bags (Stomacher 400 Classic Bags, Vicor, Serbia) and stored 9 days in air at 4±1°C.

The total viable count was determined according to the standard ISO method [13]. Results of the microbial count determinations are presented as log CFU/g of meat.

Determination of TVB-N content in marinated chicken breast fillets during storage was performed according to the method suggested by Goulas and Kontominas [14]. Results of the TVB-N content in marinated chicken breast fillets are presented as mg N/100 g of meat.

All measurements were conducted in triplicate for each sample. All parameters were described by means and standard deviation, in format $X \pm SD$. Statistical analysis of the results was conducted using GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). One-way ANOVA and post hoc Tukey's test were performed to assess the significance of differences among various groups. Values of $p < 0.05$ and $p < 0.01$ were considered significant. Multiple linear regression (MLR) was performed in JMP 10 Software (SAS Institute Inc., Cary, NC).

3. Results and Discussion

The total viable count is a microbiological parameter commonly used to determine hygiene status of the meat and contributes shelf-life of meat and meat products. The total viable counts in marinated

chicken breast fillets during 9 days of storage are presented in Table 1. During the storage period, the total viable counts gradually increased in all examined groups of marinated chicken, and significant differences ($p < 0.01$; $p < 0.05$) were found between groups on days 0, 3 and 6 of storage. At the beginning of the study (day 0), the initial total viable count was the lowest in group II marinated chicken, and the highest in group III marinated chicken.

Table 1. Mean total viable counts (log CFU/g) in marinated chicken breast fillets during storage

Marinade group	Day of storage			
	0	3	6	9
I (control)	4.11±0.44 ^a	5.33±0.51 ^A	7.03±0.24 ^A	8.86±0.51
II	3.68±0.35 ^A	5.87±0.48 ^B	7.15±0.27 ^{Ba}	8.70±0.43
III	4.73±0.31 ^{aB}	5.66±0.33 ^C	6.44±0.18 ^{AB}	8.60±0.41
IV	3.82±0.30 ^{AB}	6.03±0.67 ^{ABC}	6.74±0.18 ^a	8.95±0.30

Within a row, means with common superscript letter are significantly different ^{A-C} $p < 0.01$;

^a $p < 0.05$

Group I – water and 6% NaCl

Group II – water, 6% NaCl and 2% phosphate

Group II – water, 6% NaCl and 2% citrate

Group IV – water, 6% NaCl, 1% phosphate and 1% citrate

At the end of the study (day 9), the total viable counts in all tested groups reached values above 8 log CFU/g, and no significant differences ($p > 0.05$) were found between marinade treatments. These results are in agreement with findings from Susiluoto *et al.* [15] and Samoui *et al.* [16]. According to standard NF-V01-003, the maximum recommended limit for a total viable count in marinated chicken is 5.7 log CFU/g [11], which, in our study, was exceeded on day 3 (marinade groups II and IV) and day 6 (marinade groups I, II, III, IV).

For estimating meat freshness or shelf-life, TVB-N as a product of microbial decarboxylase activity was investigated. TVB-N values in marinated chicken were significantly higher ($p < 0.01$; $p < 0.05$) compared to those in the control, but were below the recommended value of 40 mg N/100 g of meat until day 9. The average TVB-N content in marinated chicken breast fillets during the storage period of 9 days are presented in Table 2.

Table 2. Average of total volatile basic nitrogen (TVB-N) in marinated chicken fillets during storage (mg N/100 g)

Marinade group	Day of storage			
	0	3	6	9
I (control)	20.44±1.35 ^{AC}	20.21±0.21 ^{BCD}	19.83±2.61 ^B	37.57±0.41 ^{Aab}
II	22.82±0.39 ^{aC}	23.94±0.88 ^{AC}	30.64±3.45 ^{ABC}	52.78±6.19 ^a
III	20.44±1.35 ^{aAB}	26.11±1.30 ^{ABa}	23.24±4.81 ^A	57.11±10.22 ^A
IV	21.75±0.23 ^B	24.64±0.53 ^{aD}	23.29±1.08 ^C	51.75±9.05 ^b

Within a row, means with common superscript letter are significantly different ^{A-D} $p < 0.01$;

^{a-b} $p < 0.05$

Group I – water and 6% NaCl

Group II – water, 6% NaCl and 2% phosphate





Group II – water, 6% NaCl and 2% citrate

Group IV – water, 6% NaCl, 1% phosphate and 1% citrate

On day 0 of storage, the lowest TVB-N value was in control group chicken (group I) and the highest was in group III marinated chicken. By day 6, TVB-N values in all tested groups were below the recommended value of 40 mg N/100 g. On day 9 day of storage, TVB-N values in marinated

chicken exceeded the recommended value and were statistically significant higher ($p < 0.01$ or $p < 0.05$) compared to those of the control group where the TVB-N value was below 40 mg N/100 g. Patsias *et al.* [17] found that the initial TVB-N value of 12 mg/100g sharply increased in chilled chicken fillets stored in air, resulting in high TVB-N values (49 g/100g) after 9 days of storage. In the present study, increases in the total viable count were followed by subsequent increases of TVB-N content in marinated chicken breast fillets, but this was not the case in control group chicken fillets. Khalafalla *et al.* [18] stated a clear relationship between the microbiological quality of broiler chicken breasts and level of TVB-N formation. The MLR results we obtained showed that the most significant ($p < 0.05$) increase in TVB-N occurred in chicken breast fillets treated with sodium citrate (marinade groups III and IV) where positive correlations between total viable count and TVB-N was established ($p < 0.05$). Although changes in total viable count in marinade group II showed a similar pattern as in other experimental groups, MRL analysis showed a negative correlation between these two parameters (microbial counts and TVB-N) (Table 3).

Table 3. MLR results of total viable count vs. TVB-N

Marinade group	Estimate	Std Error	t Ratio	t Ratio	Prob> t
I (control)	-0.59	1.16	-0.51		0.6159
II	-4.88	1.48	-3.31		0.0037*
III	7.36	1.34	5.50		<0.0001*
IV	3.04	1.39	2.19		0.0409*

* significance level of $p < 0.05$

Group I – water and 6% NaCl

Group II – water, 6% NaCl and 2% phosphate

Group II – water, 6% NaCl and 2% citrate

Group IV – water, 6% NaCl, 1% phosphate and 1% citrate

4. Conclusion

Sodium tripolyphosphate and sodium citrate, alone or in combination, did not inhibit microbial growth in marinated chicken breast fillets during storage. MLR results indicated that sodium tripolyphosphate slows down TVB-N formation. Moreover, it seems that sodium citrate potentiates the growth of aerobic bacteria and has a positive effect on TVB-N formation in marinated chicken breast fillets.

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