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ORIGINAL ARTICLE

The Effect of Saffron on Microbial, Physicochemical and Texture Profile of Chicken (Breast) Meat Stored in Refrigerator

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ABSTRACT

Background: Saffron roast chicken is one of the most popular and delicious foods in Iran, which is prepared from a mixture of saffron with chicken meat and then cooking the mixture. The aim of this study was to investigate the effects of saffron on the chicken meat stored at refrigerator temperature.

Methods: After mixing chicken meat with saffron powder stigma, the microbial, physicochemical and texture factors such as *Staphylococcus aureus*, Fecal coliforms, mold and yeast, pH, water holding capacity (WHC), percentage of cooking loss, lipid oxidation (TBARs) and textural profile analysis were measured. All of these experiments were carried out during the storage period.

Results: In samples treated with saffron, TBARs (Thiobarbituric acid reactive substances) were less than control samples, and other parameters had no significant difference with the control sample. The overall conclusion is based on this principle that saffron reduces fat oxidation in chicken breast meat during storage, but has no statistically significant effect compared to the control sample in relation to other microbial parameters and texture quality. No antimicrobial activity was observed due to the lack of use of saffron as an aqueous or alcoholic extract.

Conclusion: Saffron stigma powder can be considered as an improving agent of physico-chemical characteristics of chicken meat.

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Introduction

Meat is one of the best sources of protein for human consumption, and it is consumed extensively throughout the world due to its pleasant flavor and taste (1). Poultry meat has been widely used in recent decades and in many countries of the world, and various reasons such as very low production costs, rapid growth of poultry, high nutritional value, and its use in processed products have led to the consumption and popularity of this type of meat (2). Fresh chicken products are usually kept at refrigerated temperatures (2-5°C). Fat oxidation and microbial growth, and consequently physical changes during storage in the refrigerator may occur. Fresh chicken poisoning is a burden on the consumer and requires the development of new

ways to increase stability and, in general, the safety / quality of meat, which is one of the main problems of the poultry industry (3).

Food distribution and storage was developed in 1940 with the availability of low-cost home refrigerators and freezers which helped to maintain food. Then other factors, such as artificial drying processes, vacuum packaging, irradiation and chemical additives have been added. Nowadays, food consumers are concerned about preservatives in food and the general approach to the use of natural preservatives has been improved, as these substances have the susceptibility to remove microbial agents, which grow in refrigerated temperatures or anaerobic conditions (4). One of the methods of food storage is the use of plant/herb extracts, and spices. Today, due to increased consumer demand for more healthy and free of chemical preservatives, the use of natural preservatives and eco-friendly technologies have been proposed. Unlike synthetic compounds, natural preservatives obtained from plant extracts are rich in phenolic compound that can increase the overall quality of the food by reducing fat oxidation and microbial growth (5).

One of these natural preservatives is saffron. Saffron is the most expensive spice in the world, which is produced and prepared from the dried clove of Crocus sativa L., predominantly in Iran, and later in Greece, Morocco, India, Spain and Italy. Saffron is often taken into account because of its high ability to produce color, taste, and its characteristics in food aroma. These properties are essentially related to the presence of compounds such as picocrocin, safranal and crocin (6). Also, these active compounds, i.e. safranal and crocin, may be responsible for bactericidal activity in their environment, including various salmonella species, and subsequently with less probability the *Staphylococcus* and *Escherichia coli* species (6). Considering that in Iran, saffron is traditionally used to produce meat-based foods such as saffron roast chicken, and also considering the existence of ambiguities regarding the effect of saffron on microbes in the food and the improvement of physicochemical properties, this study aims to examine the effect of using saffron on microbial properties and physicochemical characteristics of chicken meat stored in the refrigerator during storage.

Materials and Methods

Sampling was taken from the whole chicken meat which was delivered in Shiraz city and by the project designers themselves. The herbarium code for the used saffron was PM832 which was ordered and purchased from the Saffron Corporation of Ghaenat Mashhad. The YGC (Yeast Extract Glucose Chloramphenicol) Agar medium was purchased from Merck company and CHROMagar™ *S. aureus* and CHROMagar™ ECC (Escherichia Coli Counts) were also purchased.

2 grams of saffron was dissolved in one liter of water, after which the stigma were removed from the liquid by passing it from a filter and then the obtained liquid was used. The sample storage containers in the refrigerator were made of white polypropylene (PP) which were sterile and a cellophane layer was applied to these containers. The microbial properties tests were performed in 5 different days (0, 3, 5, 7 and 9, days), chemical tests in 4 days (0, 2, 6 and 8, days), and physical examination or tissue testing in 3 different days (0, 3 and 6, days). It should be noted that all specimens were tested on the same day with 2 repetitions.

TBARS measurements: 1 g of the sample was mixed with 10 ml distilled water of zolal chemical company, and passed through the Wattman™ filter paper size 40. Then three different solutions were prepared for different steps of this test; solution A contained 100 ml of chloride acid 0.25 molar, solution C contained 1 gr of BHT (Butylated hydroxytoluene) in 50 cc of ethanol 96% and B solution contained 0.187 gr of TBA (Thiobarbituric acid) and 7.5 grams of TCA (Trichloroacetic acid). To carry out the test with the above solutions, at first, 1.5 ml of solution C was added to solution B, and then both of these solutions were charged with solution A up to 100 ml. Finally, 4 milliliters of the solution were vortexed with 2 milliliters of the filtered sample by shaker for 2 minutes and then were placed in boiling bain-marie at 95 ° C for 15 min.

After the solution was cooled down to the lower temperature, the solution was isolated for 10 minutes at 1000 gr at room temperature, with the K303 sigma centrifugal machine manufactured by the United States of America. Finally, the absorbance of light for the supernatant layer at 532 nm wavelength was recorded by Japan's Apel Spectrophotometer and compared with the curve derived from MDA (Malondialdehyde) measurement. TBARS was reported in mg of malondialdehyde per kg of chicken breast meat. (7).

Cooking loss measurement: the weight of the chicken breast samples was measured in the raw form and in pieces of 2 in 2 centimeters and then placed in a tray coverd by aluminum. Then placed in the oven so that the central heating temperature reaches to 75°C. Afterwards, the specimens were cooled and re-weighed at the room temperature. The difference between the initial weight of the specimens and their weight after being placed in the oven were calculated and reported as Cooking

Loss (8).

Water holding capacity measurement: (WHC) method is based on the amount of the sample water loss due to the pressure applied on it. Pieces weighing 0.5 grams were placed between two Wattman paper filters, size 40, and two glass plates, with 10 kg load was placed on the top of the plate for 5 minutes. The weight difference resulting from this process indicated the loss of sample water, and the results indicated the percentage of water released from the sample relative to its initial weight (8).

pH measurement: the samples were completely homogenized in 10 ml of distilled water, and then their pH was measured using a pH meter (Metrohm 827, made in Switzerland) (9).

Texture profile analysis: the Texture analyzer (CT3), manufactured by Brookfield USA Company was used. The dimensions of the chicken breast meat samples were 2.5×2×2. Samples were taken out of the cover and two consecutive compressions of 50% of the diameter of the chicken breast with a thickness of 2.5 cm, by using a 7 gr stainless steel probe of the TA41 model with a diameter of 6 mm was done. The speed of the pre test, the test speed and the speed of the post test were 2, 1 and 1 mm / s, respectively. The waiting time between the two test cycles was considered to be 0 seconds. The model of the fixed device base was TA-BT-KI. Data collection and computation (hardness, cohesiveness, springiness, gumminess, chewiness) were performed by using the (specialized) software (TexturePro CT, Brookfield, USA).

Microbial analysis: The count of mold and yeast was performed using the method used in the study of Alexopoulos et al. (2013) after incubation at 25 ° C for 72 hours to 120 hours. In order to count *Staphylococcus aureus* and total fecal coliforms, CHROMagar ™ *S.aureus* and CHROMagar ™ ECC chromium agar culture media were used. Pink to purple color in colonies indicated *staphylococcus aureus* and the red color indicated coliforms. The purpolitic culture medium was used and the incubation temperature was 37°C and its duration was 24 hours for all forms and 48 hours for *Staphylococcus aureus*. In addition, counting were done from plates with a colony number of 30 to 300 (10, 11).

The statistical analysis of the obtained data was performed by using Statistical Packages for Social Sciences (SPSS) Version 21.0. ANOVA was used to analyze the data. Duncan's post hoc test was used to compare the means. In the statistical evaluation, the significance level was considered to be 0.05 ($P \leq 0.05$). Also, due to the fact that the measurements of the factors were done on different days, in order to evaluate the process of changes over time, the ANOVA for Repeated Measurement Analysis was used.

Results

Figure 1 illustrated the mean and standard deviation of the data from the chemical tests during the different days for the control group and the treated group with saffron. The results showed that the pH of chicken meat in the control group and the treated group with saffron increased significantly with the increase in storage time in the refrigerator ($P<0.0001$).

Also, the rate of cooking loss in chicken breast meat at the beginning of the storage period was 0.57±0.03 (or 57%), which increased continuously in the control group with an increase in storage duration, and this increase was not statistically significant ($P>0.05$). However, in the sample treated with saffron, with increasing the duration of storage, there was a significant decrease in the amount of cooking loss ($P<0.05$) (Table 1).

However, the amount of water holding capacity of chicken meat samples at the beginning of the storage period was 0.28±0.20 (or 28%). In the control

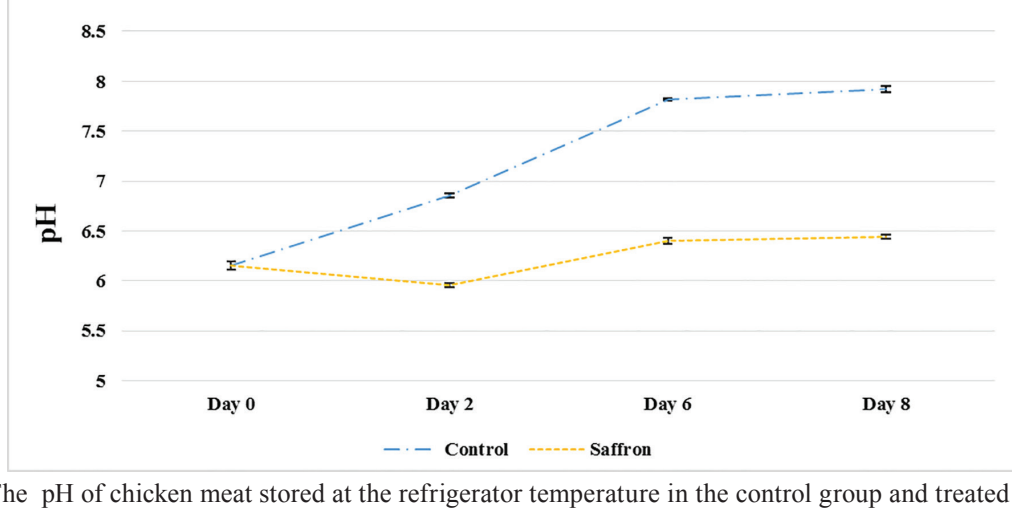

Figure 1: The pH of chicken meat stored at the refrigerator temperature in the control group and treated with saffron in different days.

sample, the highest amount was related to the water storage capacity on the second day, which was higher than the saffron treated samples (0.44±0.01), but until the end of the period, the decrease was significant (P<0.05) and the water holding capacity in the control sample on the eighth day has the lowest level, i.e. 0.34±0.04. According to Table 1. The highest WHC in the last day of storage (day 8) was related to the saffron treated sample (0.55±0.5).

Initially, the MDA value was 0.22±0.00 ppm, which was followed by a decreasing trend for the control sample as time passed until the end of the period (eighth day). At the end of the period, the highest amount of MDA for control sample was (0.26±0.02 ppm) and lowest was for saffron treated samples (0.18±0.00 ppm). According to the results of statistical analysis, the incremental rate of MDA for control sample was not significant (P<0.05), but this percentage had a significant decrease for the treated sample (P<0.05) (Figure 2).

Table 2 indicates the mean and deviation of the data criterion related to the results of texture profile measurement test during different days for the control group and treated group with saffron. At the begining, the hardness of the meat texture was 130.3±110.3, which with an increase in the storage time in the control sample, and all the treatments, there was a decrease in the hardness of the meat in comparison to the begining.

On day zero, the hardness of the meat texture was 1303±110.3, which, with an increase in storage time in the control sample, and all the treatments, there was a decrease in the hardness of the meat in comparison to the day zero. For control samples at the end of the storage period, the highest reduction in hardness was observed (248.06±06.06), which is a statistically significant decrease (P<0.01). Sample treated with saffron at the end of storage period had the least reduction in hardness (719±133.99), but for this sample, the reduction trend was considered statistically significant (P<0.05).

The level of cohesiveness at the beginning of the storage period is 0.34±0.12 and the springing effect of the samples is about 8.04±1.32. At the end of the storage period (sixth day), the changes in the springiness of the meat tissue are lower for the control sample and the treated sample. Although the elasticity of the treated sample at the end of the storage period was higher than the control sample, none of these changes were statistically significant (P<0.05).

On day zero, the amount of gumminess is 431.45±126.92 which, with increasing the storage time, on the sixth day at the end of the storage period,

Table 1: Mean and standard deviation of cooking loss and water holding capacity for chicken breast meat stored at the refrigerator temperature in the control group and treated with saffron in different days.

Test	Group	Days			
		0	2	6	8
CL	Control	[1]^{A}0.57±0.03^{a}	^{A}0.6±0.02^{a}	^{A}0.59±0.04^{a}	^{A}0.61±0.04^{a}
	Saffron	^{A}0.57±0.03^{a}	^{AB}0.56±0.01^{ab}	^{B}0.5±0.02^{b}	^{C}0.42±0.01^{b}
WHC	Control	^{A}0.28±0.02^{a}	^{B}0.44±0.01^{a}	^{AB}0.37±0.04^{a}	^{A}0.34±0.04^{a}
	Saffron	^{A}0.28±0.02^{a}	^{B}0.39±0.03^{a}	^{C}0.56±0.01^{b}	^{C}0.55±0.02^{a}

[1]Capital letters indicate a significant difference in each column and lowercase letters indicate a significant difference in each row

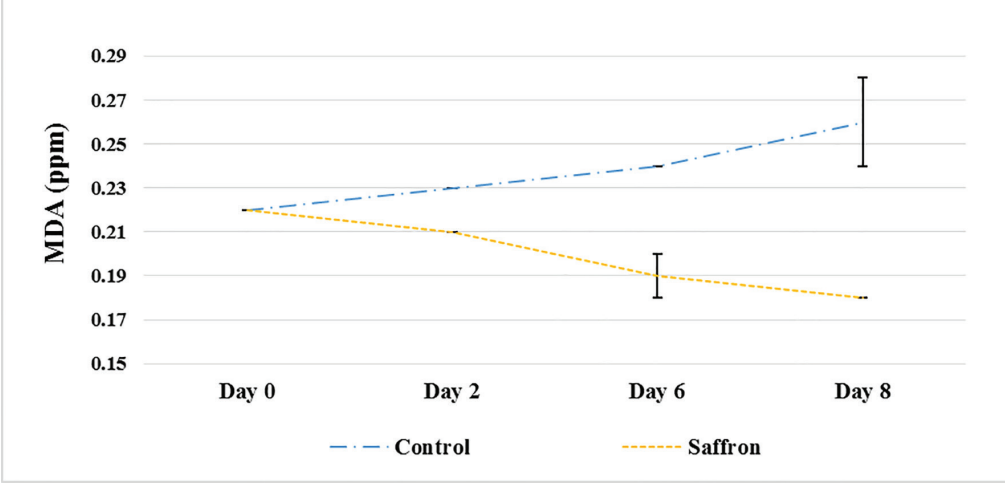

Figure 2: The amount of malondialdehyde in chicken meat stored at the refrigerator temperature in the control group and treated with saffron in different days.

the highest amount of gumminess was obtained for saffron treated (235.51±22.55) and the lowest rate of gumminess was for the control sample (94.54±36.7). On day zero, chewiness is 32.22±4.42. In the control sample, after 3 days, the highest amount was (34.71±27.81) and at the end of the period (6th day) the lowest rate of chewing ability was observed (4.18±3.21), and it is important that this decreasing trend was not statistically significant (P>0.05). Sample treated with saffron on the third day had the lowest amount of chewiness (9.58±4.19), which was followed by ascending and increasing trend by the 6th day, which was statistically significant (P<0.05).

Table 3 represents the mean and standard deviation of the data obtained from microbial tests during different days for the control group and treated with saffron. During the storage period at refrigerator temperature, the total number of fecal coliforms increased significantly in the control group and treated with saffron (P<0.0001). By the end of the storage period (ninth day), the number of staphylococcal bacteria in the chicken meat and the saffron treated sample had a significant increase (P<0.0001) and their level was 6.07±0.01 and 6.78±0.00 Log (cfu/gr), respectively, the highest was on the ninth day. According to Figure 3, the number of mold and yeasts in the day zero is 4.21±0.05. In the treated group and control group, the number of mold was significantly increased (P<0.05). The number of mold in the treated sample with saffron on the 9th day was less than that of the control sample and was 6.36±0.09, respectively.

Discussion

In the current study, in the control group the pH was increased gradually but in the treated group with saffron this index was relatively stable. This finding indicates that an increase in pH during the storage period of chicken meat is due to the production and accumulation of base compounds such as ammonia and TVB–N (total volatile basic nitrogen method) which is produced by bacteria that cause degenaration, such as proteolytic bacteria (12). The reduction in pH in carcasses is a desirable post-mortem change. Due to the fact that the acidification of the environment creates inappropriate conditions for the growth of bacteria in meat, hence, reducing the meat pH after livestock death acts as an inhibitory factor in increasing the microbial population of the meat, and is desirable. On the other hand, meat acidification affects connective tissues and, in particular, collagen fibers, and decomposes these tissues into easy gelatinous substances.

Saffron significantly (P>0.05) caused an increase in the water holding capacity of chicken meat stored in the refrigerator. In the control sample, WHC has been significantly reduced and the percentage of cooking loss has increased, but this increase in cooking loss has not been statistically significant. The main cause of the decrease in cooking and the reduction of water holding capacity in the control sample is probably due to the denaturation of proteins in the alkaline pH derived from the activity of proteolytic bacteria and the lack of protective coatings such as saffron, which increases the percentage of cooking loss (13, 14) .

A significant decreases in malondialdehyde (MDA) levels in saffron-treated specimens are probably due to the presence of natural antioxidants in saffron plants, such as phenolic safranal and crocin compounds. The inhibitory effect of safranal and crocin on the process of lipid oxidation has also been observed in other studies, such as Sanchez et al. (2012) and Asimopalo et al. (2005) (15, 16). On the other hand, Iranian researchers (2010) attributed the antioxidant effect of saffron to the presence of

Table 2: Mean and standard deviation obtained from texture profile measurement tests in chicken breast meat in different groups during storage in a refrigerator.

Test	Group	Days		
		0	3	6
Hardness	Control	[1]^{A}1303±110.3^{a}	^{B}601±0.7^{a}	^{C}248±36.06^{a}
	Saffron	^{A}1303±110.3^{a}	^{B}375±127.27^{a}	^{B}719±133.99^{ab}
Cohesiveness	Control	^{A}0.34±0.12^{a}	^{A}0.53±0.25^{a}	^{A}0.37±0.09^{a}
	Saffron	^{A}0.34±0.12^{a}	^{A}0.38±0.05^{a}	^{A}0.33±0.02^{a}
Springiness	Control	^{A}8.04±1.32^{a}	^{A}10.19±4^{a}	^{A}4.88±1.56^{a}
	Saffron	^{A}8.04±1.32^{a}	^{A}6.82±0.34^{a}	^{A}6.26±2.03^{a}
Gumminess	Control	^{A}431.45±126.92^{a}	^{A}317±153.44^{a}	^{A}94.54±36.7^{a}
	Saffron	^{A}431.45±126.92^{a}	^{AB}144.95±69.93^{a}	^{AB}235.15±22.55^{ab}
Chewiness	Control	^{A}32.22±4.42^{a}	^{A}34.71±27.81^{a}	^{A}4.81±3.21^{a}
	Saffron	^{A}32.22±4.42^{a}	^{B}9.58±4.19^{a}	^{B}14.66±6.08^{ab}

[1]Capital letters indicate a significant difference in each column and lowercase letters indicate a significant difference in each row

gallic acid and pyrogalol as bioactive compounds in saffron stigma (17). Meanwhile, the increase in the malondialdehyde level in the control sample is due to chemical degradation and the absence of inhibitory and antioxidant factors in the lipids. It should be noted that the low amount of malondialdehyde is probably due to the lack of unsaturated fats in the chicken breast meat tissue.

Reducing the hardness at the end of the storage period indicated that the chicken breast meat was softened during storage in the refrigerator, and the addition of saffron did not have a significant effect on the stability of this important feature. The oxidation of fats and the denaturation of proteins lead to the changes in muscle integrity, denaturation and accumulation of myofibrile mycobacterial proteins in chicken breast meat, which is probably due to the protective effect of saffron on the oxidation of lipids, and the treated samples have a relativly more hardness compared to the control sample. In the present study, the cohesiveness factor for the control sample has an increasing trend and for the treated samples shows a decreasing trend, which is not statistically significant, but probably because of the visual evidence of the control sample, the cohesiveness is associated with muscle contraction and drooping, which is due to the absence of lipid oxidation inhibitors, and the denaturation of proteins.

The average of the data obtained for all the samples (controlled and treated) at the end of the period and with increasing the duration of storage was less than zero, and none of these changes was statistically significant ($P<0.05$). But this result is due to the fact that the factors of gumminess and springiness are related to the hardness parameter. Several studies have clearly demonstrated the antimicrobial role of various parts of saffron (18). Incompatibility of the result of the present study with these studies is due to the way of using saffron in food tissue. Since, in these studies different parts of saffron as aqueous or alcoholic extract were used. However, in the present study, due to investigating the role of saffron powder in the surface texture of chicken (a common form of saffron used in saffron roast chicken) and the absence of the use of aqueous or alcoholic extract, there was no significant effect on the antimicrobial effects of saffron powder.

Conclusion

Saffron has indicated a positive result in improving physicochemical properties, but could not have an inhibitory effect on microbial growth. Saffron

Table 3: Mean and standard deviation of *Staphylococcus aureus* and fecal coliforms in the cheaken breast meat stored at the refrigerator temperature in the control group and treated with saffron in different days.

Test	Group	Days				
		0	3	5	7	9
Staphylococcus aureus	Control	[1]$^{A}4.34\pm0.02^{a}$	$^{B}5.41\pm0.2^{a}$	$^{C}6.46\pm0.04^{a}$	$^{D}6.73\pm0.0^{a}$	$^{D}6.07\pm0.0^{a}$
	Saffron	$^{A}4.34\pm0.02^{a}$	$^{B}5.62\pm0.03^{a}$	$^{C}6.76\pm0.03^{b}$	$^{C}6.82\pm0.01^{b}$	$^{C}6.78\pm0.0^{a}$
ECC	Control	$^{A}3.91\pm0.17^{a}$	$^{B}5.8\pm0.01^{a}$	$^{C}7.83\pm0.03^{a}$	$^{D}8.91\pm0.02^{b}$	$^{D}8.91\pm0.0^{a}$
	Saffron	$^{A}3.91\pm0.17^{a}$	$^{B}5.96\pm0.01^{b}$	$^{C}8.13\pm0.05^{b}$	$^{D}8.84\pm0.01^{a}$	$^{D}8.89\pm0.02^{a}$

[1]Capital letters indicate a significant difference in each column and lowercase letters indicate a significant difference in each row

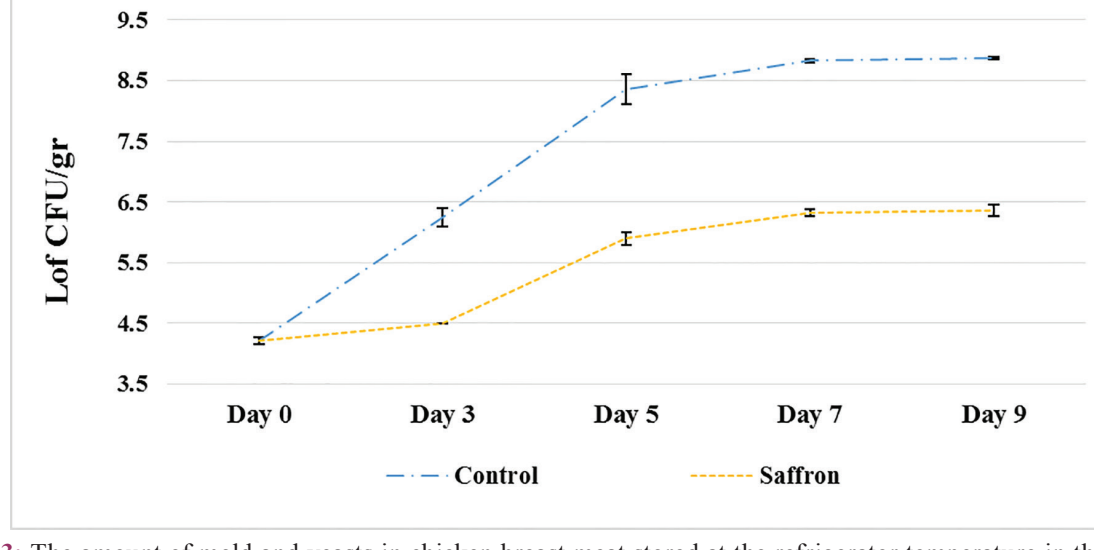

Figure 3: The amount of mold and yeasts in chicken breast meat stored at the refrigerator temperature in the control group and treated with saffron in different days.

has been shown to increase the pH of the chicken breast meat to the iso-electric point and reduce the oxidation of fats by inhibiting the production of malondialdehyde in improving the chemical and physical properties of the chicken breast meat. Due to the lack of use of saffron as aqueous or alcoholic extract no antimicrobial activity was observed. So, according to the above information, saffron powder can be used as a substance for improving the physico-chemical properties in different food matrices.

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Conflict of Interest

None declared.

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