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Usage of concentrated pomegranate to extend the shelf life of chicken breast

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ABSTRACT

Chicken meat is commonly marketed at refrigerated temperatures (2-5ºC). The major concern for retailers and consumers is the quality and safety of refrigerated chicken meat. During chilling period, chicken meat undergoes many undesirable changes due to microbial growth that lead to spoilage and economic loss. Therefore, this study was conducted to evaluate the effect of pomegranate juice (PJ) in three concentrations (1, 2, and 4%) on sensory attributes, chemical and microbiological quality of chicken breast stored at 4±1ºC for 12 days. The results showed that dipping of chicken breast meat samples in PJ at three concentrations 1, 2 and 4% can improve storage stability of chicken breast samples. This study also concluded that the use of PJ at a concentration of 4% is more effective compared to concentrations of 1% and 2%. Therefore, PJ could be used as a natural antioxidant and antimicrobial preservative for chilled chicken meat held at refrigerated temperature.

INTRODUCTION

Chicken meats continue to be the most significant foods consumed worldwide, itis a good source of protein with high biological value, vitamin A, thiamine, iron, phosphorus and nicotinic acid (**Koblitz, 2011**). Fresh meats are highly perishable and support the growth of pathogenic and spoilage microorganisms. Despite applying many controls and preventive measures, food-borne illnesses are still an important public health issue in both developing and non-developing countries (**Zhou et al. 2010).**

The fresh meat is very sensitive to spoilage by microbial growth and oxidative reactions. High level protein and moisture cause microbial spoilage of meat while the aerobic condition induces oxidation of lipid and protein. Decreasing microbial growth and delaying lipid and protein oxidation during storage can increase the shelf-life of meat (**Vaithiyanathan et al. 2011**).

It is known that chicken meat is a reservoir for a large number of bacteria that may be pathogenic to human. Typically, these occur at low levels of sanitation and may pose a threat to the consumer if the product is not treated in a safe manner (**El-Fakhrany et al. 2019**). The bacterial

contamination and hygienic measures during meat production can be measured using the aerobic plate count and total Enterobacteriaceae (**Hamed et al. 2015**).

Nowadays, synthetic preservatives are being applied to prevent the microbial growth and as well as retarding the oxidation reactions in meat. The consumers are unsatisfied from various chemical preservatives because of their side effects such as carcinogenicity and teratogenicity. The excessive demand for natural preservatives results in their extended utility (**Giatrakou and Savvaidis 2012**).

Acidification using organic acids and natural acidic fruit juices such as pomegranate juice is an extensively used method in food processing to extend the shelf-life (**Sengun and Karapinar, 2004**).

The pomegranate (Punicagranatum) is a well-known source of important nutrients. Because of its great nutritious value, antioxidant capacity, bioactive components, and consumer appeal, it is referred to be a "superfruit." It includes hydrolysable tannins, condensed tannins, flavonoids, anthocyanins, and phenolic and organic acid components, all of which have been linked to a variety of health advantages (**Nuncio-Jáuregui et al. 2015**). The edible part of the fruit contains considerable amount of acids, sugars, vitamins, polysaccharides, polyphenols and important minerals (**Vardin and Fenercioglu, 2003**).

Pleasant flavor of pomegranate juice results from the combination of various taste, aroma and mouth feel sensations. The distinguished taste is due to mainly the presence of sugars (glucose and fructose) and organic acids (primarily citric and malic acids) **(Vázquez Araújo et al. 2011)**. The great antioxidant potency from different components of pomegranate fruit such as juice, peel and seeds have been discovered **(Gil et al. 2000)**.

The antioxidant activity of pomegranate juice is higher than other fruit juices and beverages (**Seeram et al. 2008**). This antioxidant activity is correlated to the high level of phenolic compounds, including anthocyanins (3-glucosides and 3,5-diglucosides of delphinidin, cyanidin, and pelargonidin), ellagic acid, punicalin, punicalagin, pedunculagin and various flavanols (**Alighourchi et al. 2008).**

Therefore, the objective of this study was toinspect effect of dipping of chicken breast meat in different concentrations of pomegranate juice solution (1%, 2% and 4% v/w) on the shelf life and sensory attributes of chicken meat stored at 4ºC for 12 days.

MATERIALS AND METHODS

Collection of samples:

The chicken meat samples were obtained from a local poultry slaughterhouse in Benha and Damanhur province, wrapped in a sterile polyethylene bags and transported to the laboratory in isolated boxes with cooling packs.

Chicken meat samples that have been proven to contain E. coli, Pseudomonas and Staphylococcus aureus will be used to study the effect of pomegranate juice (PJ) in three concentrations (1, 2, and 4%) on these microbes during storage at 4±1ºC for 12 days.

Preparation of pomegranate juice (PJ)

Fresh pomegranate fruits (*Punicagranatum*) were obtained from a local supermarket. The fruits were washed and cut into four pieces. The seeds/arils were manually separated and ground in amixer for 30 s and then passed through muslin cloth. After filtering by a Millipore filter with a 0.22 µm nylon membrane under vacuum at 25 °C, the freshly prepared PJ was sterilized by the high-pressure treatment and was stored at 4°C until use, no more than 24 h later according to **Bazargani-Gilani et al. (2015).**

Preparation of chicken meat samples according to Vaithiyana than et al. (2011)

Twelve samples of chicken breast proved to be contaminated with E. Coli $<10^2$ cfu/g were divided into two groups; treated and control one. The treated groups were divided into 3 groups that were dipped in pomegranate juice (PJ) at concentration 1%, 2% and 4% for 15 minutes and then drained well for 5 minutes on a sterile stainless wire mesh screen. The same technique was applied for samples of chicken breast proved to be contaminated with pseudomonas and Staphylococcus aureus. Chicken

breast were individually sealed in clean polyethylene bags and stored at 4 °C for up to 12 days. The treated and control samples stored at 4±1°C and examined regularly every 3 days at (zero, 3rd, 6th, 9th and 12th) for sensory, chemical and microbiological parameters.

Sensory analysis according to Lawless and Heymann (2010).

Fifteen panelists individual (adult, untrained) were asked to assess the sensory qualities of chicken breast samples. The samples were blind–coded with special codes; the panelists were not informed about the experimental approach. They were asked to give a score for each of overall acceptance while the samples were fresh (uncooked). Nine-point descriptive scale was used. A score of 7–9 indicated ''very good'' quality, a score of 4.0–6.9 ''good'' quality, a score of 1.0–3.9 indicated as spoiled was used for the evaluation of appearance, tenderness, and flavor.

Chemical analysis of treated chicken meat:

Measurement of pH according to (ES 63-11/2006) was verified using a pH-meter (Digital, Jenco 609). The pH was measured by blending 10 g sample with 90 ml deionized water for 2 min. The pH of the obtained suspension was measured with a digital pH meter.

Measurement of Thiobarbituric acid reactive substance (TBARS) according to (ES 63/9-2006). Ten grams of the sample were blended with 48 mL distilled water. Two ml of 4% ammonium chloride (to bring the pH to 1.5) was added to the previous contents in a warring blender for 2 min and left at room temperature for 10 min. The mixture was quantitatively transferred into Kjeldal flasks by washing with an additional 50 mL distilled water, followed by an antifoaming preparation and a few glass beads. The Kjeldal distillation apparatus was assembled and the flask was heated to 50 °C. Distillates were collected at 10 min from the time of the boiling commencing. The distillates (50 mL) were mixed, and then were pipette into a glass Stoppard tube. Then, 5 mL TBA reagent (0.2883/100 mL of glacial acetic acid) was added, the tube was stoppered, shacked and immersed in a boiling water bath for 35 min. A blank was similarly prepared using 5 mL distilled water with 5 mL of TBA reagent and treated like the sample. After heating, the tube was cooled under tap water for 10 min. A portion was transferred to a curette and the optical density (D) of the sample was read against the blank by means of a spectrophotometer (Perkin Elmer, 2380, USA) at a wave length of 538 nm. The TBA value (mg malondialdehyde /Kg of sample) = Dx7.8 D: the read of sample against blank.

Measurement of total volatile basic nitrogen (TVBN) according to method recommended by (**ES 63/10-2006)**.Teng of the samples was mixed with 100 ml distilled water and washed into a distillation flask with 100 ml distilled water; then 2g of magnesium oxide and an antifoaming agent were added. The mixture was distilled using the micro Kjeldahl distillation apparatus. Distillate was collected for 25min into 25 ml 4% boric acid and five drops of Tashero indicator. The solution was titrated using (0.1 M) HCl to calculate the total volatile basic nitrogen in the sample in terms of mg VBN/100g meat

Microbiological analysis:

Preparation of serial dilution according to APHA (1992).

Chicken breast samples were firstly cauterized by using hot spatula (surface sterilization) then the cauterized parts were removed by using sterilized scalpel and forceps, then under complete aseptic conditions 25 grams of each sample were weighted and transferred into a sterile homogenizer flask contained 225 ml of (0.1%) peptone water. The content of each flask were homogenized at 14000 rpm for 2.5 minutes for obtaining a dilution of 10^{-1}, from which 1 ml was transferred with a sterile pipette to a sterile test tube containing 9 ml of (0.1%) peptone water, from which a decimal serial dilution were prepared in a sequential manner up to 10^{-10}, to cover all expected range of samples contamination. For microbial counts, colonies were counted and recorded in colony forming units per gram (cfu/g) of meat sampled using the formula:

cfu/g = level of dilution plated x number of colonies counted/volume plated.

These were further expressed in mean colony forming units pergram (mean cfu/g) and converted to $\log_{10}$ base values ($\log_{10}$cfu/g).

2.6.2. Total aerobic bacterial count was determined according to **FDA, 2001**using Plate count agar (pour technique) and was incubated for 48 ± 2 h at 35°C.

Enumeration of E.coli were determined according to **ISO (16649-2:2001).** Total E.colicount was carried out by pour plate method on TBX medium and incubated for 18 hours at 44ºC. E. coli produced blue colonies; the colonies were counted and expressed as CFU/g.

Pseudomonades were determined according to **ISO 13720:2010** using pseudomonas agar base supplemented with cetrimide, fucidin, and cephaloridine, incubated spread plates at 25°C for 48 hours then examine for growth and fluorescence at 24 and 48 hours, using both white and UV light.

Staphylococcus aureus count were determined according to **FDA (2001)** on Baird Parker agar plate at 35°C for 48 hours, suspected colony appeared as black, shiny colonies with halo zone around them were picked up for morphological examination and biochemical identification.

Statistical Analysis:

Triplicate samples (n = 3) were analyzed for each property. The results were expressed in terms of mean and stander deviation (SD) of mean. The means were compared by One Way ANOVA followed by Duncan's Multiple Range Test **(Duncan, 1955)** using SPSS software version 17.0. Differences between means were determined by the least significant difference test, and significance was defined at $P<0.05$.

RESULTS

Table(1) Mean scores of sensory characteristics of chicken breast treated with different concentration of pomegranate juice during chilling storage at 4°C for 12 days.

Descriptor	Sensory scores				
	Day 0	Day 3	Day 6	Day 9	Day 12
	Appearance				
Control	5.81 ± 0.02^{a}	5.62± 0.04^{a}	Spoiled	Spoiled	Spoiled
1%	6.03 ± 0.04^{a}	5.84± 0.02^{a}	5.42± 0.27^{a}	5.42±0.26^{a}	Spoiled
2%	6.02± 0.02^{a}	5.87± 0.03^{a}	5.85 ± 0.32^{a}	5.53±0.22^{a}	4.54 ± 0.33^{b}
4%	6.14± 0.03^{a}	6.04± 0.04^{a}	6.00± 0.26^{a}	5.84± 0.24^{a}	5.64± 0.26^{a}
	Tenderness				
Control	5.34 ± 0.02^{a}	5.33± 0.04^{a}	Spoiled	Spoiled	Spoiled
1%	5.65 ± 0.04^{a}	5.55± 0.02^{a}	5.43± 0.27^{a}	5.13± 0.26^{a}	Spoiled
2%	5.58 ± 0.02^{a}	5.46± 0.03^{a}	5.37 ± 0.32^{a}	5.23± 0.22^{a}	4.26 ± 0.33^{b}
4%	5.82 ± 0.03^{a}	5.32± 0.04^{a}	5.49± 0.26^{a}	5.44± 0.24^{a}	5.34± 0.26^{a}
Flavor					
Control	5.80 ±0.03^{a}	5.14± 0.04^{a}	Spoiled	Spoiled	Spoiled
1%	6.53±0.04^{a}	6.42± 0.02^{a}	5.62± 0.27^{a}	5.15± 0.26^{a}	Spoiled
2%	6.36±0.02^{a}	6.24± 0.03^{a}	5.65 ± 0.32^{a}	5.53± 0.22^{a}	5.34 ± 0.33^{a}
4%	6.32±0.03^{a}	6.22± 0.04^{a}	5.72± 0.26^{a}	5.54 ± 0.24^{a}	5.47± 0.26^{a}

Data given as mean ± SD of 3 replicates.

Table (2) Pattern of pH of chicken breast treated with different concentration of pomegranate juice during chilling storage at 4°C for 12 days:

Chicken breast	pH values				
	Day 0	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	5.45±0.06[a]	5.96±0.12[a]	6.10±0.01[a]	6.20±0.01[b]	6.42±0.02[c]
1%	5.44±0.04[a]	5.94±0.01[a]	5.96±0.06[a]	6.12±0.06[b]	6.28±0.06[b]
2%	5.42±0.03[a]	5.92±0.02[a]	5.95±0.06[a]	6.10±0.06[b]	6.15±0.01[b]
4%	5.45±0.03[a]	5.90±0.06[a]	5.90±0.06[a]	6.00±0.19[a]	6.10±0.06[b]

Data given as mean ± SD of 3 replicates.
Values with different letters within the same row differed significantly at (P<0.05).

Table (3): Pattern of TBARS values (MDA mg/kg) of chicken breast treated with different concentration of pomegranate juice during chilling storage at 4°C for 12 days.

Chicken breast	TBARS values (malonaldehyde mg/kg meat)				
	Day 0	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	0.45±0.03[a]	0.68±0.06[b]	0.95±0.05[c]	1.21±0.01[d]	1.86±0.03[e]
1%	0.44±0.02[a]	0.59±0.04[a]	0.76±0.04[a]	0.91±0.06[a]	1.14±0.04[a]
2%	0.43±0.03[a]	0.48±0.03[a]	0.61±0.02[a]	0.75±0.02[a]	0.91±0.06[a]
4%	0.41±0.01[a]	0.43±0.03[a]	0.45±0.02[a]	0.56±0.03[a]	0.78±0.03[a]

Data given as mean ± SD of 3 replicates.
Values with different letters within the same row differed significantly at (P<0.05).
Es (1090/2005) stated that TBARS values should not excess 0.9 mg/kg

Table (4) Pattern of TVN values (mg/100g) of chicken breast treated with different concentration of pomegranate juice during chilling storage at 4°C for 12 days**.**

Chicken breast	TVBN values (mg/100 g meat)				
	Day 0	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	6.5±0.12[e]	12.43± 0.33[d]	18.10±0.34 [c]	20.58±0.64 [b]	23.80±0.31 [a]
1%	6.38±0.28 [e]	11.63±0.16 [d]	15.33±0.11 [c]	18.53±0.40 [b]	20.46±0.28 [a]
2%	6.35±0.24 [e]	10.23±0.23 [d]	14.41±0.28 [c]	15.22±0.41 [b]	18.92±0.48 [a]
4%	6.25±0.15 [c]	9.43±0.20 [d]	12.53±0.32 [c]	13.41±0.44 [b]	15.72±0.22 [a]

Data given as mean ± SD of 3 replicates.
Values with different letters within the same row differed significantly at (P<0.05).
Es (1090/2005) stated that TVN values should not excess 20 mg/100 gm.

Table (5) Pattern of aerobic bacterial count (log_{10}cfu/g) in chicken breast treated with different concentrations of PJ during chilling storage period at 4±1°C for 12 days

Chicken breast	Total aerobic bacterial count (log_{10}cfu/g)				
	Day 0	Day 3	Day 6	Day 9	Day 12
Control	3.86±0.45^{a}	4.83±0.16^{a}	5.92±0.24^{b}	6.73±0.19^{c}	7.75±0.19^{c}
1%	3.70±0.26^{a}	3.86±0.32^{a}	4.31±0.35^{a}	4.74±0.14^{b}	5.15±0.27^{b}
2%	3.65±0.21^{a}	3.73±0.18^{a}	4.22±0.29^{a}	4.48±0.22^{a}	4.56±0.22^{b}
4%	3.62±0.35^{a}	3.65±0.14^{a}	4.07±0.15^{a}	4.31±0.20^{a}	4.39±0.25^{a}

Data given as mean ± SD of 3 replicates.
Values with different letters within the same row differed significantly at *(P<0.05).*

Table (6) Pattern of E.coli count (log_{10}cfu/g) in chicken breast treated with different concentrations of PJ during chilling storage period at 4±1°C for 12 days**.**

Chicken breast	E.coli count(log_{10}cfu/g)				
	Day 0	Day 3	Day 6	Day 9	Day 12
Control	1.74±0.16^{a}	1.81±0.12^{a}	1.97±0.11^{b}	2.23±0.14^{c}	2.48±0.12^{c}
1%	1.42±0.12^{a}	1.63±0.10^{a}	1.72±0.16^{a}	1.84±0.12^{b}	1.98±0.14^{b}
2%	1.28±0.14^{a}	1.47±0.12^{a}	1.53±0.14^{a}	1.62±0.10^{a}	1.79±0.11^{b}
4%	1.16±0.11^{a}	1.25±0.11^{a}	1.39±0.12^{a}	1.45±0.15^{a}	1.51±0.13^{a}

Data given as mean ± SD of 3 replicates.
Values with different letters within the same row differed significantly at *(P<0.05).*

Table (7) Pattern of Pseudomonads (log_{10}cfu/g) in chicken breast treated with different concentrations of PJ during chilling storage period at 4±1°C for 12 days.

Chicken breast	Pseudomonads count (log_{10}cfu/g)				
	Day 0	Day 3	Day 6	Day 9	Day 12
Control	1.61±0.10^{a}	1.86±0.13^{a}	1.94±0.17^{b}	2.36±0.16^{c}	2.71±0.14^{c}
1%	1.33±0.11^{a}	1.33±0.13^{a}	1.57±0.10^{a}	1.67±0.10^{b}	1.79±0.14^{b}
2%	1.15±0.11^{a}	1.19±0.10^{a}	1.25±0.10^{a}	1.32±0.17^{a}	1.43±0.12^{b}
4%	1.09±0.06^{a}	1.10±0.08^{a}	1.12±0.12^{a}	1.19±0.11^{a}	1.24±0.12^{a}

Data given as mean ± SD of 3 replicates.
Values with different letters within the same row differed significantly at *(P<0.05).*

Table (8) Pattern of *Staphylococcus aureus* count (log_{10}cfu/g) in chicken breast treated with different concentrations of PJ during chilling storage period at 4 ± 1°C for 12 days.

Chicken breast	*Staphylococcus aureus* count (log_{10}cfu/g)				
	Day 0	Day 3	Day 6	Day 9	Day 12
Control	1.26±0.04[a]	1.63±0.01[a]	1.93±0.07[b]	2.76±0.07[c]	2.87±0.08[c]
1%	1.22±0.08[a]	1.52±0.03[a]	1.82±0.06[a]	1.94±0.07[b]	2.15±0.10[b]
2%	1.15±0.08[a]	1.33±0.07[a]	1.55±0.05[a]	1.72±0.08[a]	1.84±0.08[b]
4%	1.06±0.07[a]	1.19±0.10[a]	1.35±0.08[a]	1.38±0.04[a]	1.69±0.13[a]

Data given as mean ± SD of 3 replicates.
Values with different letters within the same row differed significantly at *(P<0.05).*

DISCUSSION

Sensory Evaluation:

Sensory profile allows us to evaluate the quality of food and in some time to identify unwanted contaminants (**Rasooli 2007**). It is obvious from the results obtained in **Table (1)** that in the freshly chicken breast samples (day 0), the panelist found that both treated samples and untreated samples with PJ fared well in all sensory attributes.

According to the results of sensory evaluation, samples with pomegranate juice had the higher scores than control ones in all of sensory attributes. There was significant variation ($P < 0.05$) in some of the tested parameters. Addition of pomegranate juice at 1, 2, and 4 % significantly improved appearance, tenderness and flavor of chicken breast till the end day9 of storage especially concentration 4%. Sensory properties were enhanced till the end day 9 of storage by addition of pomegranate juices.

The sensory quality of chicken breast samples, especially of the control sample, reduced remarkably from day 6 of storage and became unacceptable to be cooked due to change of odour. Sensory attributes changes were less pronounced in chicken breast samples containing 4% PJ, in comparison with control samples and other chicken breast samples contain different concentrations of PJ.

These results agree with **Salem et al. (2020)** who reported that pomegranate molasses enhanced sensory properties in chicken meat served at a university student hostel. **Bazargani-Gilani et al. (2015)** concluded that pomegranate juice improves desirable sensory attributes of chicken meat including taste, color, odor, texture and overall acceptability. In addition, pomegranate juice, not only give appropriate color and flavor to foods but also, they can extend the shelf-life of foods. Therefore, pomegranate juice products are one of the most popular flavorings used to give flavor to several foods such as chicken, fish, salads and appetizers in Iran and Turkey (**Karabiyikli and Kisla, 2012**).

Chemical analysis of treated chicken meat:

Hydrogen ion concentration (pH)

The obtained results in **Table (2)** showed that pH values of control and treated samples with PJ increased during the storage period till end of day 12th. The increasing of pH may be due to the action of endogenous or microbial enzymes such as protease and lipase that cause an increase in volatile bases (e.g., ammonia and trimethylamine) during prolonged storage (**Chaijan et al. 2005**). In addition, there was a statistically significant difference between the control and treatment groups at day 12. The present results are in agreement with **Obuzand Cesur (2009)** found that the chicken breast meat dipped in pomegranate juice presented the lowest pH value.

4.2.2. Thiobarbituric Acid Reactive Substances (TBARs)

Thiobarbituric acid reactive substance (TBARS) assay is one of the most widely used methods for measuring secondary oxidation products mainly Malondialdehyde (MDA), which are known as the cause of oxidative rancidity, which may contribute to the off flavor

of oxidized fat **(Zhang et al. 2016).**

The recorded data in **Table (3)** revealed that the mean values of TBA in control samples were increased from 0.45 ± 0.03 at zero day of storage to 1.86 ± 0.03 mg MDA/kg at day 12 of storage. Treated chicken breast with PJ at 1%, TBA values increased from 0.44 ± 0.02 mg MDA/kgat zero day of storage to 1.14 ± 0.04 mg MDA/kg at day 12 of storage. At 2 % of PJ,TBA values increased from 0.43 ± 0.03 mg MDA/kg at zero day of storage to 0.91±0.06MDA/kg at day 12 of storage. Finally, chicken breast treated with 4% PJ, TBA values increased from 0.41 ± 0.01 mg MDA/kg at zero day of storage to 0.78 ± 0.03 mg MDA/ kgat day 12 of storage. Irrespective of treatment, TBARS gradually increased with increase in storage period, TBA values in treated samples significantly reduced malondialdehyde (MDA) values, as compared to the control one. In general, lipid oxidation of control and treated chicken samples was low and below 0.5 mg MDA/kg showing no oxidative rancidity during the storage period.

According to **ES 1651/2005** stated that TBA should not excess 0.9 mg/kg meat Samples are considered valid for consumption until the day 3 in control group and until the day 6 in the case of PJ 1%. Samples treated with PJ 2 and 4% are valid for consumption until day 9 and 12 of storage respectively.

Treated groups with Pomegranate juice showed decreased lipid oxidation significantly ($P<0.05$) as compared to the control. The large amount of phenolics contained in PJ may cause its strong antioxidant ability. The present results were in agreement with previous studies **(Naveena et al. 2008** and **Vaithiyanathan et al. 2011)** who reported that pomegranate juice reduce protein and lipid oxidation**.** In addition, **Devatkal et al. (2011)** reported that pomegranate powder was more effective in reducing TBARS formation. Therefore, it was concluded that extracts of these fruits by-products could be successfully added to meat to act as antioxidant.

Total Volatile Nitrogen (TVN)

Results in **Table (4)** revealed that, the examined control samples have a higher increasing rate in TVN content which was 6.5 mg/100 g at zero time of cold storage and continued to increase to reach 23.80 mg/100 g after 12 days. On other side, the corresponding value for the chicken breast samples that were dipped in pomegranate juice (PJ) at concentrations 1%, 2% and 4% had the lowest TVN content from the beginning of cold storage (6.38, 6.35 and 6.25 mg/100 g) until the end of cold storage period after 12 days (20.46, 18.92 and 15.72 mg/100 g) for concentration 1, 2 and 4% of pomegranate juice, respectively. The increase in TVN value in the meat might be attributed to the breakdown of protein because of activity of different microorganisms and their proteolytic enzymes **(Hassan and Omama, 2011).** Positive effect of addition of pomegranate juice may be due to inhibition of microorganism and preventing of protein breakdown resulting in volatile nitrogen compounds. These results declared especially by high concentrations of pomegranate juice 4%.

According to permissible limits established by **ES/ 1651 (2005)** which stated that TVN should not exceed 20 mg/100 g, control group still fit for consumption until day 6of storage, treated chicken meat samples with PJ 1% still valid for day 9 of storage while chicken breast treated with 2 and4% PJ which do not exceed the permissible limit till day 12 of storage at 4^{o}C, respectively and become fit for consumption.

The obtained results were matched with **Bazargani-Gilani et al. (2015)** who reported that pomegranate juice2%in chicken breast meat significantly lowered the TBARS, and protein oxidation in treated sample compared to control. In addition, pomegranate juice reduces the lipid peroxidation and oxidative stress by directly scavenging the free radicals **(Banihani et al. 2013).**

Microbiological Examination of treated chicken meat with pomegranate juice solution:

Total aerobic bacterial Count:

According to **ES 1651/2005** stated that total bacterial count should not excess 10^5/g. On the day 3, the APC for control samples was

4.83±0.16, which was close to the maximum limit of APC recommended by (**ES 1651/2005**), while on the day 6, the APC of control samples was 5.92±0.24, which exceeded the maximum recommended limit indicating shelf-life is less than 6 days for the untreated control chicken breast samples. The APC values for the samples treated with PJ 1% still valid for consumption till day 9 of storage, while when the concentration of PJ was increased to 2% and 4% the treated samples exhibited a delayed growth for APC till day 12 and greater reducing effect in total bacterial count was noticed in PJ 4% increasing the shelf-life for these samples to 12 days during chilling storage. This result was supported by **Bazargani-Gilani et al. (2015)** who reported that pomegranate juice 2% has significant effect on decreasing total viable count of chicken breast meat during refrigerated storage. Treated chicken samples with pomegranate juice at concentrations 2% and 4% does not exceed the permissible limit 10^5cfu/g even after a storage for 12 days. This could be due to the antimicrobial action of PJ components especially condensed tannins and protein perceptible phenolics. The phenolics inhibited the microbial growth in samples treated with PJ, by protein binding or enzyme inhibition (**Kumar and Vaithiyanathan, 1990).**

E.coli count:

Mentioned results in **Table (6)** showed that mean value of E.coli counts were increased in control samples from 1.74±0.16at zero day to 2.48±0.18log10 cfu/g at day 12 of storage. Treated chicken breast with pomegranate juice at concentration 1, 2 and 4%, E.coli count was slightly increase (from 1.42±0.12 at day zero to 1.98±0.18log10 cfu/g at day 12 of storage), (from 1.28±0.14 at day zero to1.79±0.11log10 cfu/g at day 12 of storage), and (from 1.16±0.11at day zero to1.51±0.13log10 cfu/g at day 12 of storage), respectively. Treatment with pomegranate juice at different concentration produced significantly decrease in E.coli count when compared to control sample. Similarly, other researchers have reported that PJdecreased Escherichia coli count significantly in treated poultry meat **(Dahham et al. 2010 Salam et al. 2011 Bazargani-Gilani et al. 2015 Daoutidou et al. 2021).**

Pseudomonas count:

It is now well established that Pseudomonas spp. may form a significant part of the spoilage microflora of chicken meat stored under refrigeration (**Jay et al. 2005**). Pseudomonas spp. are known to compete other bacterial groups (Gram-positive or Gram-negative) for nutrients by forming siderophores, that may inhibit growth of both spoilage microorganisms and pathogens (**Wei et al. 2006**). Proteolysis is an important phenomenon involved in the meat spoilage. The microflora of chicken meat particularly pseudomonads are responsible for proteolysis and the subsequent slimeproduction. This event starts when the bacterial counts reach 10^7-10^8 cfu/g and the contents of glucose and gluconate are exhausted (**Nychas and Tassou 1997**).

The above-mentioned results in **Table (7)** revealed that initial pseudomonas spp. count was 1.61±0.10 $\log_{10}$cfu/g increasing during storage to reach final population 2.71±0.14 $\log_{10}$ cfu/g (control samples), whereas treated samples with pomegranate juice at concentration 1, 2 and 4% respective counts were 1.79±0.14, 1.43±0.12 and 1.24±0.12$\log_{10}$ cfu/g at day 12 of storage lower than the values in the control samples. Pseudomonas spp. population in all treatments were significantly ($P<0.05$) lower than control samples.
From the previously mentioned data it was found that PJ 2% and 4% were the most significantly ($P<0.05$) effective treatment in reducing the Pseudomonas count. These results were supported by **Bazargani-Gilani et al. (2015)** who reported that treatment with pomegranate juice significantly decrease Pseudomonas species in treated chicken breast.

Staphylococcus aureus count (SC):

From results given in **Table (8)** the Staphylococcus aureus count for control samples was 1.26 ± 0.04, 1.63 ± 0.01, 1.93 ± 0.07, 2.76 ± 0.07 and 2.87 ± 0.08 log10 cfu/g at zero, 3, 6, 9 and 12 days, respectively.

Staphylococcus aureus count was 1.22 ± 0.08, 1.15 ± 0.08 and 1.06 ± 0.07 log10 cfu/g for PJ 1%, 2% and 4% treated samples at day zero, respectively and this indicated that increase concentration of PJ on chicken breast significantly ($P<0.05$) lowered the Staphylococcus aureus

count.

The Staphylococcus aureus count underwent incremental increases during day 12 of storage for all examined samples. By the day 12 the Staphylococcus aureus count was 2.15±0.10, 1.84±0.08 and 1.69±0.13log10cfu/g for PJ 1%, 2% and 4% treated samples, respectively. However, significantly lower staphylococcus count ($P<0.05$) was recorded for treated samples with pomegranate juice at concentration 1%, 2% and 4% stored during the entire storage period under refrigeration, higher reduction was observed in treated samples with 4% PJ.

Addition of pomegranate juice significantly decrease Staphylococcusaureus count. These results are in agreement with **Malviya et al., (2014)** who studied antibacterial activity of pomegranate peel extracts and found that the maximum was against Staphylococcus aureus. Also, **Tayel et al. (2012)** reported that decontamination of meat surfaces can be achieved by addition of pomegranate extracts. Moreover, **Kanatt et al. (2010)** who studied the antibacterial& antioxidant properties of pomegranate extract and showed a noticed antibacterial effect to Staphylococcus aureus.

Generally, it is recommended that these natural components such as pomegranate juice should be incorporated in our food not only due to their antimicrobial effect but also to enhance the nutritive value of food to achieve healthy food.

CONCLUSION

It can be concluded that the pomegranate juice has the ability to delay microbial and chemical changes, extend the shelf-life and exhibit desirable sensory attributes including taste, color, odor, texture and overall acceptability in chicken breast meat. Therefore, considering the consumer preference for natural additives, pomegranate juice can be used as a natural antioxidant, antimicrobial, flavoring, texturing and coloring additive in chicken breast as well as other kinds of meat products.

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