Original Article

Evaluation of Red Grape Seed Essential Oil Nanoemulsion (*Vitis Vinefera*) on the Shelf Life of Fresh Packaged Chicken Fillets during Refrigerated Storage at 4 °C

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Abstract

In this study, red grape seed essential oil (GSEO) and it,s nanoemulsion form (GSEON) with the average diameter of 124 nm were used, in order to increase the shelf life of chicken fillets at cold storage (4+1 °C) during period of 14 days. The results of chemical tests showed that PV and TVN values were significantly lower in the GSEON 5% treatment compared to other treatments (P≤0.05). TVC reached 7 logCFU/g in control treatments after 6 days, however, TVC was in standard range in the another treatments, especially the GSEON treatment, after 14 days. The *Staphylococcus aureus* and PTC counts in the GSEON 5% treatment during storage was significantly lower than other treatments (P≤0.05). It can be concluded that, use of GSEON especially in 5% concentration to reduce spoilage of organisms and delay the oxidation of lipid and finally, increasing the shelf life of chicken fillet storage at 4 °C was recommended.

Keywords: Chicken fillet, *Vitis vinefera* essential oil, Nanoemulsion, Shelf life

Introduction

Oxidation and changes in the color and texture of the chicken meat caused the limited period of storage and approximately 25% of poultry primary production lost due to microbial and chemical spoilage [1]. Lipid oxidative causes unpleasant odor, undesirable changes in taste and reduced nutritional value of the product while microbial contamination lead to loss of product and serious dangers in consumer health.

There is a growing trend for using of natural preservatives with antibacterial and antioxidant activity to improve the quality, extend the shelf life and prevent the economic losses of poultry products [2]. Different essential oils and plant extracts such as grape seed extract [3], bearberry extracts [4], tomato plant extract [5], Zataria mulrifolra [1], rosemary, cloves [6] and Pistacia atlantica essential oil [7], had been used to increase the shelf life of refrigerated meat and delay the microbial and chemical spoilage during storage.

Grapes (Vitis vinifera) belong to Vitaceae family is one of the most important fruits of the world, with an annual production of about 60 million tons [8]. Grape seed extract have phenolic compound including catechin, epicatechin, gallic acid and proanthocyanidins with high antimicrobial and antioxidant properties [9, 10].

Nanoemulsion is one of the newest technologies that have been emerged in the food industry to product materials with high quality and increase the shelf-life [11]. Phenolic compound of GSEO are unstable and influenced by the different parameters such as temperature, pH, light, oxygen, astringent and bitter taste [12], so using of nanoemulsion (GSEON) form of GSEO could protects the above mentioned compounds, protect products against oxidation during storage, avoid undesirable odor and taste and prevent the loss of nutritional loss in the products. Sogut and Can Seydim, (2018) study has shown that coating grape seed extract with chitosan can enhance the antimicrobial and antioxidant properties of the GSEO, as well as increasing the shelf-life of chicken breast fillets stored at 4 °C. Given the economic and

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How to cite this article: Ghanbari, M., Motallebi, A., Rokni, N., Anvar, A. Evaluation of Red Grape Seed Essential Oil Nanoemulsion (*Vitis Vinefera*) on the Shelf Life of Fresh Packaged Chicken Fillets during Refrigerated Storage at 4°C. Arch Pharma Pract 2020;11(S1):119-27.

nutritional value of chicken and temporary maintenance methods, it seems to be essential the survey on quality and shelf life in the refrigerator affected various herbal additives such as GSEO especially in nanoemulsion form. So, in the current study, essential oil and nanoemulsion form (GSEON) of grape seed were used to increase the shelf life of chicken fillet stored at 4 °C for 14 days.

Materials and Method

Preparation of essential oil from grape seed (GSEO)

Grape seeds were purchased from a local pharmacy (Tehran, Iran). Grape seeds were dried at room temperature (in a dark room), powdered and subjected to hydrodistillation using a Clevenger-type apparatus for 6h at 25 °C according to the method recommended in the British Pharmacopoeia [13]. Anhydrous sodium sulfate was used to dehydrate the essential oil. The essential oil was stored in dark bottle at 4 ºC until next study.

Preparation of grape seed essential oil nanoemulsion (GSEON)

The method of producing nanoemulsion from GSEO was spontaneous emulsification that recommended by Bouchemal et al. (2004) [14]. Polyoxyethylene sorbitan monovalote was considered as an organic and Tween 80 and GSEO were also selected as the oil phase. The speed addition of organic phase to oil phase with a magnetic stirrer (6500 rpm, 15 min) was fixed in all treatments. In this study, pH of mixtures adjusted on 7.4 with phosphate buffer. Finally, the nanoemulsion form of GSEO was dried by a freeze dryer.

Size and morphological characteristics of nanoemulsion

Morphology of dry GSEON was confirmed with a scanning electron microscopy (SEM, Philips XL30, Netherland).

Also, particle size distribution and zeta potential was determined in pH 8.0 using Zetasizer Nano ZS (Malvern Instruments, England).

Chicken sample preparation

Chicken samples were purchased from the local market (Sari, Mazandaran provinces, Iran) and transported to laboratory in ice boxes. Filleting were immediately (50±5 g) done in 1 hour under sterile condition.

Preparation of Chicken fillets samples

Chicken fillets samples were divided into 5 treatments as follow: control samples (without any GSEO), GSEO 1%, GSEON 1%, GSEON 2% and GSEON 5%. Chicken fillets immersion in solution containing GSEO and GSEON solution for 1 min, and then, packed, labeled, and stored at 4 °C for 14 days. In this research, the effect of the aforementioned forms on chemical parameters [peroxide value (PV) and total volatile basic nitrogen (TVB-N)] and microbial parameters [total viable count (TVC), pscycrotrophic count (PTC) and Staphylococcus aureus of chicken fillets kept at 4 °C in the refrigerator from zero to 14 days (days 3, 5, 7 and 14) was evaluated.

Chemical analyses

Three chicken fillets were randomly selected and tested for evaluation of chemical and microbiological analysis to determine the overall quality of chicken fillets. All measurements were carried out in triplicate.

Measurement of peroxide value

Peroxide value (PV) was measured according to the AOAC method (AOAC, 2005). The sample (20 g) was weighed in a 220-ml glass and heated in a water bath at 60 °C for 3 min, then thoroughly agitated for 3 min with 30 ml acetic acid–chloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered under vacuum through whatman paper. Saturated potassium iodide solution (0.5 ml) was added to the filtrate, which was transferred into titrator equipped with stirrer and pH electrode. The titration was allowed to run against standard solution of sodium thiosulfate (25 g/l). PV was calculated and expressed as milliequivalent peroxide per kg of sample:

$$PV\ (meq / kg) = (S \times N)/W \times 1000$$

Where: S is the volume of titration (ml), N the normality of sodium thiosulfate solution (N =0.01), and W the sample weight (kg).

Determination of total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) was determined according to the method that described by Jeon et al (2002).

Microbiological analyses

Total viable count (TVC) were determined by inoculating 0.1 ml of the sample homogenate onto triplicate sterile plates of dried Tryptic Soy Agar (Merck, Darmstadt, Germany) using the surface spread technique, then the plates were incubated for 48 h at 37 °C [15].

Psychrotrophs count (PTC) value were determined on Tryptic Soy Agar using the surface spread technique, then the plates were incubated for 7 °C at 7 days [15]. Staphylococcus aureus were determined in Baird-Parker Agar (BPA, Merck, Germany) incubated at at 37 °C for 48 h (ICMSF, 1978). All counts were expressed as log cfu/g and performed in triplicate.

Statistical analysis

The obtained data were subjected to one-way analysis of variance using SPSS statistical software, release 20.0. Duncan's new multiple range test (at the 95% level) was performed to determine the significant differences of the

means at the 5% probability level (P< 0.05). All experiments were carried out in triplicate.

Results

The results obtained from Scanning Electron Microscope (SEM) showed that the morphology of dry nano essential oil was confirmed by this Moore Electron Microscope. The particle diameter of all samples was in nanometer scale, and in this investigation, the measured particle diameter ranged from 51.4 to 228 nm and the mean of particles` diameter was measured as 124 nm (Figure 1, and Figure 2). In the present study, the size of particles ranged from 166.8 to 291.2 nm, the dispersion indices ranged from 51.4 to 228 nm, the dispersion index ranged from 0.09 to 0.23 nm and the encapsulation production efficiency was 54.2%, which confirmed the production of homogeneous nano particles.

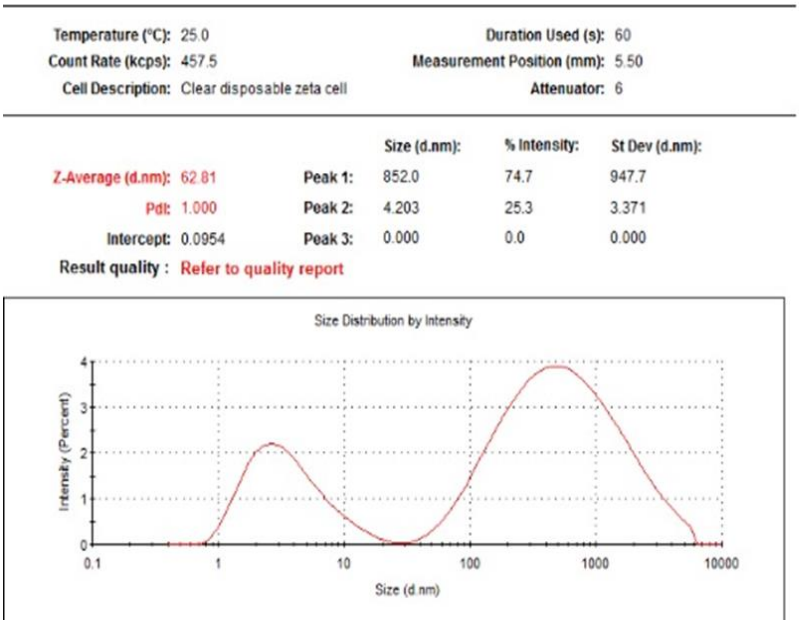

Figure 1: Diagram of Particle Sizer Device

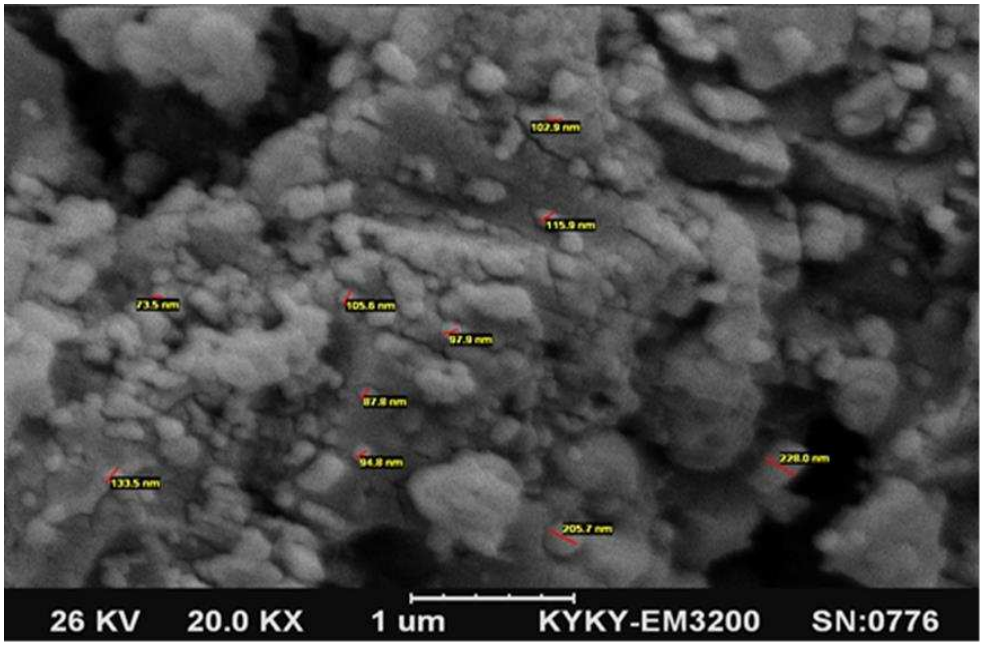

Figure 2: Scanning Electron Microscope Image of Nanocapsules of Grape Seed Essential Oil

The result of T-test with two independent samples about the difference of the impact of nano essential oil of grape seed on chicken fillet at 4 °C and 8 °C temperatures showed that since the significance level of the test for all sensory factors (odor, appearance, color, taste and overall acceptability) was considered less than the error ratio (P <0.05), with 95%

confidence level, the sensory changes of the nano essential oil of grape seed on the chicken fillet were different at two temperatures of 4 °C and 8 °C, which indicated the existence of a significant difference in the mean of data collected at these two temperatures. Likewise, the results obtained for the experiments at 5-day, 7-day, and 14-day were also true, in a way that considering the fact that the significance level of the test for all sensory factors (odor, appearance, color, taste, and overall acceptability) was lower than the considered error ratio (P <0.05), with 95% confidence level, sensory changes of the nano essential oil of grape seed on chicken fillet at 5-day, 7-day, and 14-day were different. This indicated the existence of a significant difference in the mean of data collected on these days.

The results of T-test with two independent samples about the effect of grape seed nano essential oil concentration on chicken fillet shelf life time showed that considering that the significance level of the test for all sensory factors (odor, appearance, color, taste and overall acceptability) was lower than the considered error ratio (P<0.05), with 95% confidence level, the sensory changes (mean) of the effect of grape seed nano essential oil on chicken fillet in 2 experiments of control and 1% nano essential oil are different, and indicated the existence of a significant difference in the mean of data collected in these two experiments. Likewise, the results obtained from the experiments at 5-day, 7-day, and 14-day were also true, in a way that considering that the significance level of the test for all sensory factors (odor, appearance, color, taste, and overall acceptability) was lower than the considered error ratio (P<0.05), with 95% confidence level the sensory changes (mean) of the effect of grape seed nano essential oil on chicken fillet in experiments at 5-day, 7-day, and 14-day were different, indicating the existence of a significant difference in the mean of data collected on these days.

The results obtained in this study about this hypothesis that the grape seed nano essential oils impacts on the total microbial load and the number of cryogenic bacteria and Staphylococcus aureus microbe and the ratio of TVN and PV in chicken fillet, showed that the grape seed nano essential oils had an antimicrobial property in chicken fillet. Investigating the difference of the means of each one of the factors using ANOVA test showed that since the significance level was lower than the error ratio (P <0.05) in all sample days (3, 5, 7, 14), there was a significant difference at 95% confidence level (Table 1) between the ratio of TVC experiments with various nano essential oils. Therefore, the Tukey test was used to exactly specify the ratio of TVC in what percentage of the nano essential oils has been lower or higher. For this purpose, the mean of nano essential oils experiments was divided into three general groups, the mean of TVC in the 5% nano essential oil sample had the lowest mean (3.800) and the control sample had the highest mean (6.100).

Table 1: ANOVA Test Results (The Effect of Nano Essential Oil on TVC in Terms of Various Days)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TVC.3day	Between Groups	6.338	4	1.584	9.867	.014
	Within Groups	.803	5	.161		
	Total	7.141	9			
TVC.5day	Between Groups	9.309	4	2.327	7.458	.025
	Within Groups	1.560	5	.312		
	Total	10.870	9			
TVC.7day	Between Groups	16.576	4	4.144	9.177	.016
	Within Groups	2.258	5	.452		
	Total	18.833	9			
TVC.14day	Between Groups	16.461	4	4.115	8.229	.020
	Within Groups	2.501	5	.500		
	Total	18.962	9			

As it is observed in Table 2, considering that the significance level was lower than the error ratio (P <0.05) in all sample days (3, 5, 7, 14), there was a significance difference at 95% confidence level between the ratio of PTC experiments with various nano essential oils percentage. Thus, the Tukey test was used to specify exactly the ratio of PTC in what percentage of the nano essential oils has been lower or higher (Table 2). For this purpose, the mean of nano essential oils experiments was divided into three general groups, that the mean of PTC in the 5% nano essential oil sample had the lowest mean (3.995) and the control sample had the highest mean (6.305).

Table 2: ANOVA Test Results (The Effect of Nano Essential Oil on PTC in Terms of Various Days)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PTC.3day	Between Groups	6.690	4	1.673	12.702	.008
	Within Groups	.658	5	.132		
	Total	7.349	9			
PTC.5day	Between Groups	7.909	4	1.977	7.110	.027
	Within Groups	1.391	5	.278		
	Total	9.299	9			
PTC.7day	Between Groups	19.090	4	4.772	11.141	.010
	Within Groups	2.142	5	.428		
	Total	21.232	9			
PTC.14day	Between Groups	15.267	4	3.817	6.099	.037
	Within Groups	3.129	5	.626		
	Total	18.396	9			

As it is observed in Table 3, considering that the significance level was lower than the error ratio (P <0.05) in all sample days (3, 5, 7, 14) a significance difference at 95% confidence level was observed between the ratio of Staphylococcus aureus experiments with various nano essential oils percentage. Thus, the Tukey test was used to specify exactly the ratio of Staphylococcus aureus in what percentage of the nano essential oils has been lower or higher. For this purpose, the mean of nano essential oils experiments was divided into three general groups, that the mean of Staphylococcus aureus in the 5% nano essential oil sample had the lowest mean (0.925) and the essential oil sample had the highest mean (5.195).

Table 3: ANOVA Test Results (The Effect of Nano Essential Oil on Staphylococcus Aureus in Terms of Various Days)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
STA.3day	Between Groups	24.347	4	6.087	27.963	.001
	Within Groups	1.088	5	.218		
	Total	25.435	9			
STA.5day	Between Groups	29.501	4	7.375	48.062	.000
	Within Groups	.767	5	.153		
	Total	30.268	9			
STA.7day	Between Groups	33.579	4	8.395	20.661	.003
	Within Groups	2.032	5	.406		
	Total	35.610	9			
STA.14day	Between Groups	30.263	4	7.566	17.222	.004
	Within Groups	2.196	5	.439		
	Total	32.459	9			

As it is observed in Table 4, considering that the significance level was higher than the error ratio (P >0.05) in all sample days (3, 5, 7), there has been no significance differences at 95% confidence level between the ratio of TVN experiments with various nano essential oils percentage. For experiments with 14-day samples, considering that the significance level was lower than the error ratio (P<0.05), no significance differences at 95% confidence level was observed between the ratio of TVN with various nano essential oils percentage. Thus, the Tukey test was used to exactly specify the ratio of TVN in what percentage of the nano essential oils in 14-day experiments has been lower or higher. For this purpose, the mean of nano essential oils experiments was divided into two general groups, in which the mean of TVN in the 5% nano essential oil sample had the lowest mean (31.040) and the control sample had the highest mean (83.335).

Table 4: ANOVA Test Results (The Effect of Nano Essential Oil on TVN in Terms of Various Days)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TVN.3day	Between Groups	5.883	4	1.471	1.077	.456
	Within Groups	6.830	5	1.366		
	Total	12.713	9			
TVN.5day	Between Groups	71.779	4	17.945	.961	.501
	Within Groups	93.331	5	18.666		
	Total	165.110	9			
TVN.7day	Between Groups	639.704	4	159.926	4.320	.070
	Within Groups	185.102	5	37.020		
	Total	824.805	9			
TVN.14day	Between Groups	3809.545	4	952.386	32.414	.001
	Within Groups	146.912	5	29.382		
	Total	3956.456	9			

As it is observed in Table 5, considering that the significance level was higher than the error ratio (P >0.05) in all sample days (3, 5, 7,14) no significance differences at 95% confidence level was observed between the ratio of PV experiments with various nano essential oils percentage.

Table 5: ANOVA Test Results (The Effect of Nano Essential Oil on PV in Terms of Various Days)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PV.3day	Between Groups	.312	4	.078	.906	.525
	Within Groups	.431	5	.086		
	Total	.742	9			
PV.5day	Between Groups	1.549	4	.387	.339	.842
	Within Groups	5.715	5	1.143		
	Total	7.263	9			
PV.7day	Between Groups	3.417	4	.854	.953	.505
	Within Groups	4.484	5	.897		
	Total	7.901	9			
PV.14day	Between Groups	1.498	4	.374	.706	.621
	Within Groups	2.652	5	.530		
	Total	4.149	9			

Discussion

The results obtained from Scanning Electron Microscope showed that the diameter of the particles was in the range of 51.4 to 228 nm, which was similar to the nanocapsule particles diameter of the olive leaf extract reported by Kesente et al. in 2017 [16]. The amount of PV peroxide number increased significantly during the study period that a significant decrease was finally observed at 5% nano essential oil compared to the other treatments. These results were similar to the researches` results of Sogut and Can Seydim in 2018 that in investigating the effect of chitosan

edible films and the grape seed extract on the quality of packaged chicken fillets in the vacuum concluded that chitosan coating along with grape seed extract could increase the chicken fillet shelf life at refrigerator temperature due to its antimicrobial and antioxidant properties. The increase in the amount of TVN-B in chicken fillets during the shelf life at 4°C was probably due to the breakdown of amino acids by the bacteria. The antimicrobial effect of grape seed essence oil on proteolytic bacteria can reduce the production of volatile amines and following it TVB-N. This finding was consistent with the findings of Katalinic et al. in 2010. Also in a similar study by Dzomba et al. in 2014, they stated that the beef meat including the leaf plants` extract reduced the creation of peroxidation and proliferation of microbes and increased the shelf life time of the beef meat [17]. Also a similar study by Carpenter et al. in 2007 showed that the addition of grape and barberry extract to raw pork meat reduced the oxidation of fat on days 9 and 12 of storage compared to the control group. Balamatsia et al. reported in 2006 that the acceptable TVB-N level in chicken meat was 30 meq/ kg, and the TVB-N level reached the standard level after 7 days in control treatment, while the TVB-N level reached the standard level after 14 days at the 5% nano essential oil treatment of grape seed [18]. The results of the present study showed that the shelf life time of chicken fillets in the control group increased from 6 days to 14 days at the 5% nano essential oil treatment of grape seed, which could be related to the decrease of the number of bacteria or their oxidative ability to eliminate amines from the nitrogen-containing non-volatile compounds when 5% nano essential oil were used. These results were consistent with the investigations of Katalinic et al. in 2010 [19].

The results obtained from the present study showed that during the storage time the Total Viable Count (TVC) of bacteria increased significantly in all treatments, but in the 5% nano essential oil treatment, it was significantly lower than other treatments. These results were in line with the findings of Sogut and Seydim in 2018, who stated that chitosan edible films along with grape extract reduced mesophilic aerobic bacteria and coliforms` counts in fillets stored at 4°C. Alves et al. in 2018 also showed that chitosan film with grape seed extract and carvacrol microcapsule in salmon fish fillet stored at 4 °C temperature of refrigerator could reduce the number of mesophilic bacteria after 7 days compared to the control group [20]. Katalinic et al. in 2010 showed that the high antimicrobial activity of grape seed extract was related to the phenolic compounds responsible for antimicrobial activity and affecting cell walls and microbial growth reversal. These results were consistent with the results of the present study. In another similar study, the effect of alginate coating with pomegranate peel essential oil on microbial characteristics of chicken fillet was investigated by Mostafa Pirahesh et al. in 2017 [21]; according to the results, by increasing the time, the total amount of bacteria in all treatments increased and this increase was more in the control treatment. By adding alginate and increasing the concentration of preservative, the amounts of total and cryogenic bacteria decreased. Alginate coating containing 1.5% pomegranate peel essential oil could be used as a natural preservative in meat products.

According to the results of the present study, the pattern of PTC cryogenic bacteria increase in all treatments was similar to the changes in total bacteria count. In a similar stud,y Zhang et al. in 2016 stated that rosemary and clove extract in chicken raw meat significantly decreased microbial growth including the number of Enterobacteriaceae and Pseudomonas during the storage in the refrigerator at 4°C. Alves et al. in 2018 also stated that chitosan film with grape seed extract and carvacrol microcapsule in salmon fish fillet stored in refrigerator at 4°C could decrease the growth ratio of cryogenic and Pseudomonas bacteria after 7 days compared to the control group. Shan et al. in 2007 showed that phenolic compounds of various plants, such as grape seed extract, could limit the effects of various foodborne pathogens [22].

A similar study was conducted by Reisi et al. in 2012 on the antibacterial effect of carboxymethyl cellulose coating enriched with grape seed extract and Thyme [23]; the results obtained in this study was similar to the results of the present study and showed that the Thyme essential oil in combination with grape seed extract could significantly reduce the number of bacteria and delay the spoilage of samples and increase the shelf life time of meat products. Al-Habib et al. in 2010, worked on the antibacterial effect of grape seed extract on Methicillin-Resistant Staphylococcus Aureus (MRSA) and concluded that all Methicillin-Resistant Staphylococcus Aureus were sensitive to this extract. The grape seed extract is full of strong polyphenolic compounds of antioxidant that can show the antibacterial activity.

Conclusion

In general, the results obtained from the present study showed that using nano essential oil of grape seed could increase shelf life time of packaged chicken fillet. The results obtained from the present study showed that the nano essential oil of grape seed could decrease the rate of increase of Nitrogenous Organic Bases (TVB-N) and Peroxide Number (PV) during the storage at 4°C by the gradual release of the constituent compounds. Total Viable Count (TVC) of bacteria, PTC cryogenic bacteria count and Staphylococcus aureus count in 5% nano essential oil of grape seed were significantly lower during the storage period than other treatments. Therefore, the use of nano essential oil, especially at 5% concentration is suggested to reduce microorganism spoilage, and delaying lipid oxidation and eventually increasing the shelf life time of stored chicken fillets in the refrigerator at 4°C.

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