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Influence of Modified Atmosphere Packaging and Multi-layer Flexible Pouches on pH of Fresh Ostrich Meat

Nazanin Zand[1]*, Ali Hafez pour[2]

[1,2] Department of Food Science and Technology, Faculty of Agriculture, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran
[*]Corresponding E-mail: n_zand2008@yahoo.com

ABSTRACT

The influence of different concentrations of two kinds of gas mixture (carbon dioxide & nitrogen) ,and vacuum conditions and packaging without gas injection and three types of flexible multilayer films in order to increase shelf life of fresh ostrich meat in the refrigerator (T= 4˚ C) were studied. Normal conditions as a control samples were compared with 2 types of gas compositions {(%30 CO_2 and %70 N_2) & (%30 N_2 and %70 CO_2)}, and also vacuum conditions. Fresh ostrich meats were packed with flexible films, {3-layer: PET (12) / AL (12) / LLD (100); 4-layer: PET (12) / AL (7) / PET (12) / LLD (100), and 3-layer: PET (12) / AL (7) / LLD (100)}. Packed samples experienced changes in pH at different times during 15 days ,with 12 treatment, 3 run, statistical analysis and comparison of data, were done by software SAS (Ver:9/1) and Duncan's new multiple range test, with confidence level of 95% (P <0.05). The shelf life of fresh samples, according to pH changes, under the gas compositions 1 and 2 and vacuum conditions in 4-layer were evaluated 15, 13 and 10 days, in 3-layer (AL: 12) were 14, 10, 7 days, with 3-layer (AL: 7) were 10, 7 days and under vacuum conditions were 6 days. As could be seen the best storage conditions of samples belonged to the 4-layer under 70% CO_2 which extended the shelf life of fresh ostrich meat up to 15 days. Packages of samples had better protective properties with 4-layer than two other containers, since steam permeability of 4-layer was less than 3-layer, and increasing CO_2 could be affected pH by producing more carbonic acid in order to maintain long-term shelf life of fresh ostrich meat.

Key words: Modified atmosphere packaging (MAP), fresh ostrich meat, pH, flexible multi-layer films (3-layer and 4-layer)

INTRODUCTION

Ostrich meat is introduced as a new alternative red meat due to low fat and high unsaturated fatty acids. Fresh Ostrich meat is usually chilled before sale 24-48 hours after death or immediately is packed by vacuum condition [1, 3, 13]. Prepared Packages of meat as fresh meat are preferred to frozen ostrich meat (crushed and ground) [1, 14]. Modern techniques of meat packaging are considered to maintain the microbial contamination and appearance of the product [25-28]. The shelf life of products can be increased by inhibiting or retarding the growth of undesirable flora. This can be achieved by manipulating the environment of meat packaging [17]. Vacuum and modifying atmosphere packaging (MAP) are techniques, which can be used in the food industry to extend shelf-life of food products [4, 5, 11]. Modified atmosphere packaging (MAP) is also a useful technique for various researches [15, 25-28]. As, chemical, enzymatic and microbial activities are controlled so that the major risks that may occur, are avoided or reduced [6, 15, 25-28]. The Ability of modified atmosphere packaging has been known to extend the shelf life of food over 100 years ago [6, 9]. Ostrich meat without efficient processing is a potential source of pathogenic microorganisms, such as aerobic and anaerobic bacteria and Salmonella , other hands a pH close to neutral (pH 5- 6) and the appropriate water activity cause the packaged ostrich meat become an ideal environment for microbial spoilage [2,16]. Although, heating and freezing meat affect microorganisms effectively, it deactivates proteins and some other physiological layers and thus

creates undesirable changes in flavor, texture, and nutrients content in packed fresh ostrich meat [9, 25-28]. In the meantime, researchers are searching for ways to deliver the chilled and fresh eat with desirable quality to the consumers in addition to increase the shelf life [12, 15, 25-28]. However, modified atmosphere packaging (MAP) which is a non-thermal method for food storage and deactivates microorganisms is widely used to prolong the shelf life and improve the quality of perishable food stored in the fridge temperature [12, 17]. Carbon dioxide, nitrogen and oxygen are generally used in these systems. All mentioned in this study include the initial CO2 / N2 concentration (%) in the head space of pouches as the independent variable for the gas atmosphere demonstrated that CO2 exerts as an antimicrobial effect in the water-phase of the food product [4,5,29-32], therefore except the effect of intrinsic, extrinsic and processing parameters on the CO2 solubility, the concentration of dissolved CO2 in the water-phase of the food product should be incorporated in this study as independent variable [12,17]. Nitrogen (N2) is a non-reactive gas that has no smell or taste, unlike carbon dioxide, is not absorbed in food or water [17, 29, 30]. It is used as a filler gas to replace oxygen and thus prevent spoilage or to replace carbon dioxide and prevent package collapse [17, 25-28]. Other hand in this study, flexible multilayer films are used for packaging fresh ostrich meat is plastic films laminated with aluminum [18-20]. These laminated packages with some metal component can considerably change the food temperatures and also microwave transparent with a high melting point instead of can [18, 24]. The most common packages that have been tried, are individual pouches made of microwave transparent rigid films such as polyethylene (LLD) , and polyethylene terephthalate (PET) , which are barrier films ,and aluminum foil [18-24]. The aim of this study is to compare the quality fresh ostrich meat under the effects of modified atmosphere packaging with gas compositions (%30 N2 and %70 CO2), (%70 N2 and %30 CO2) and vacuum, and the usage of 3 types of multilayer flexible pouches [27,29-32] for packaging fresh ostrich meat during storage times (15 days). We want to prove MAP can substitute thermal processing in conservation industries, and control pH variable [18, 25-28].

MATERIALS AND METHODS

Preparation of fresh ostrich meat

Ostrich fresh meat (10 kg weight) were chosen for this experiment bought from local supermarket in Mashhad -Iran. These samples were washed and cut to slices (7cm *15 cm). Temperature was controlled in order to decrease to ambient temperature (T=25 ° C). Samples were ready for packaging. Pouches contained 100 g, ostrich fresh meat [20, 21,23-25]. Analytical parameters such as pH (Crison 2001 pH meter; Crison Instruments, SA, Barcelona, Spain) soluble solid content (Atago RX-1000 refract meter; Atago Company Ltd., Japan), were measured according to the ISIRI regulation [7, 8, 20-23, 25-28].

Modified Atmosphere Packaging

Henkelman packing machine, model Boxer-200A was used in this project. Samples were placed into three multilayer flexible containers {4-layer: PET (12) / AL (7) / PET (12) / LLD (100); 3-layer: PET (12) / AL (12) / LLD (100) , and 3-layer: PET (12) / AL (7) / LLD (100)} for packaging. Samples were ready for gas injection and then the gas compositions (%30 N2 and %70 CO2), (%70 N2 and %30 CO2) and vacuum were transferred from modified atmosphere packaging machine. After packaging, samples were put in refrigerator immediately at 4 ^{0}C, for evaluation pH in different conditions [15, 25-28].

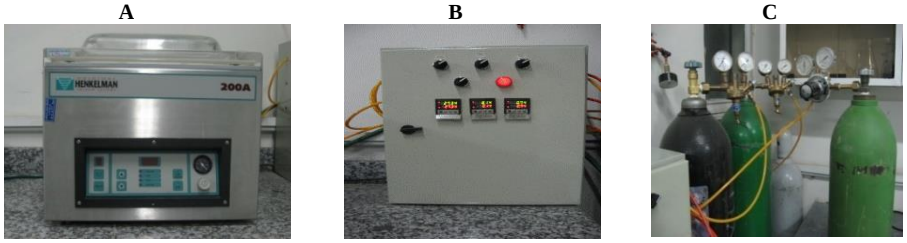

Fig 1. (A) Modified atmosphere packaging, (B) gas analyzer, (C) gas flash tank (Model: Boxer-200A)
[15, 25-28]

Chemical tests

Measurements: pH

PH meter was adjusted with a buffer solution to 4 -7 .Sample (100 g) was uniformed ,and poured into 100 ml Erlenmeyer flask , pH has been measured at ambient temperature (T=25 ° C) [7,8,15,25-28].

Samples packaging and storage

All pouches (unprocessed and processed samples), were put at refrigerator temperature (T= 4° C). Ostrich meat were packaged into 3 types of multilayer flexible films. Analytical characteristics of these barrier containers were shown in table 1 [15,19-23,25-28].

Table 1- Analytical characteristics of containers [15,19-23,25-28]

Sample	Layers	Thickness (µ)	Tensile of sealing film (N)	O.T.R ($ml/m^2.day$)	W.V.T.R ($g/m^2.day$)
PET/AL/LLD	100/12/12	124	58.88	0	0.11
PET/AL/LLD	100/7/12	119	48.89	0	0.50
PET/AL/PET/LLD	100/12/7/12	131	61.03	0	0.089

PET: Poly Ethylene Terephthalate; LLD: Low Density Poly Ethylene; AL: Aluminum

Statistical Analysis

In order to describe the variables of this experiment, we must design a model to analysis relationship between type of samples, type of treatments in different storage times on pH variable. Statistical analysis of data, was performed by software Statistical Analysis System (SAS 9/1) with ANOVA test, and comparison of data was done by Duncan's new multiple range test, with confidence level of 95% (P <0.05) [15, 25-28].

RESULTS

Total amount of pH in different conditions

According to analysis of variance table 1, the effect of different layers, gas compositions, and times and the double interactions (layer, gas), (layer, time), and (gas,time), and the triple interactions (layer, gas, time) on pH were significant (p< 0/01). The primary effects of layers, gas compositions and times on pH were significant at %1. According to analysis of variance table 1, as you observed, the coefficient of variation (CV) was in the range of 1-2 (CV = 1.565), which means we had high accuracy trials and low errors.

Table 2- Analysis of variance mean squares traits in response to treatments

Resource (Variable)	Fredom Degree	Total amount of pH
Container	2	**0.876
Gas Composition	3	**1.788
Container * Gas Composition	6	**0.0019
Time	3	**0.455
Container * Time	6	**0.002
Gas composition * Time	9	**0.319
Container * Gas Composition * Time*	18	**0.0174
Error	98	0.0003
Coefficient of Variation (CV)	-	1.565

*- **, * and ^{ns}, significance at 1% and 5% and non-significance, respectively*

Table 3- Different gases, multi-layers and storage times

Gas	Number	Layer	Number
CO2 70% and N2 30%	1	3-layer (Al:12)	1
CO2 30% and N2 70%	2	4-layer (Al:7)	2
Vacuum	3	3-layer (Al:7)	3
Control packaging 2	4	Glassy container	4
Control packaging 1 (initial day)			

According to evaluation of pH, the lowest pH were observed in control packaging 1 as an initial control. Although the highest amount were observed in control packaging 2, after 15 days, and then in vacuum and 30%CO2 conditions, which had not significant differences, as you see in figure 2. The lowest amount of pH belonged to condition under 70% CO2+30% N2.

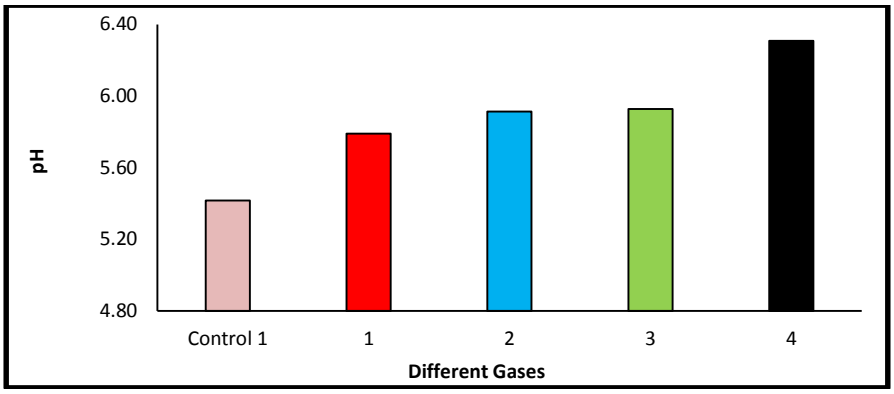

Fig 2-The effect of different concentrations of gas on pH (Single interaction)
{1:CO2 70% + N2 30%; 2:CO2 30% + N2 70%; 3: Vacuum; 4: Control}

According to evaluation of pH, the lowest pH were observed in control packaging 1 as an initial control (glassy container). However the highest amount were observed in 3-layer (AL:7 µ) , after 15 days and , then in 3-layer (AL:12 µ), as you see in figure 3 .The best condition of packaging belonged to 4-layer, which had not significant differences with 3-layer (AL:12 µ).

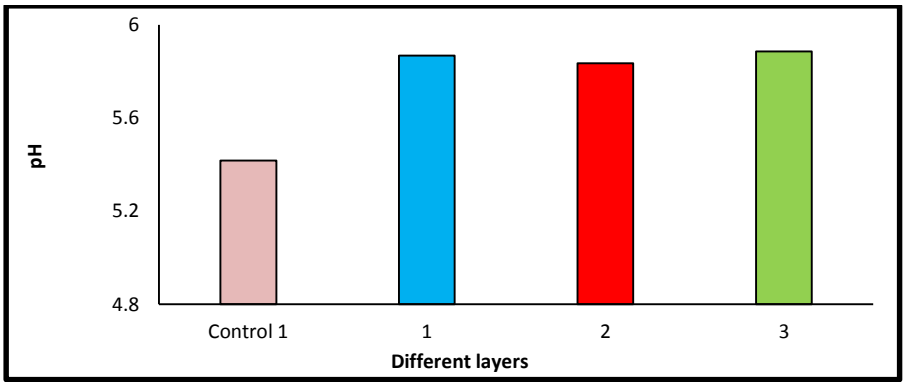

Fig 3-The effect of different layers on pH (Single interaction)
{1: 3- layer (AL: 12 µ); 2:4-layer; 3: 3- layer (AL: 7 µ); 4: Control 1}

As you see in figure 4, evaluation of pH were reported, the lowest pH in initial day as a control 1. But the highest amount were observed, after 15 days. This model ($y = - 0.00061x^2 + 0.21 x+5.44$) was described the relation between time and amount of pH, which had significantly level.

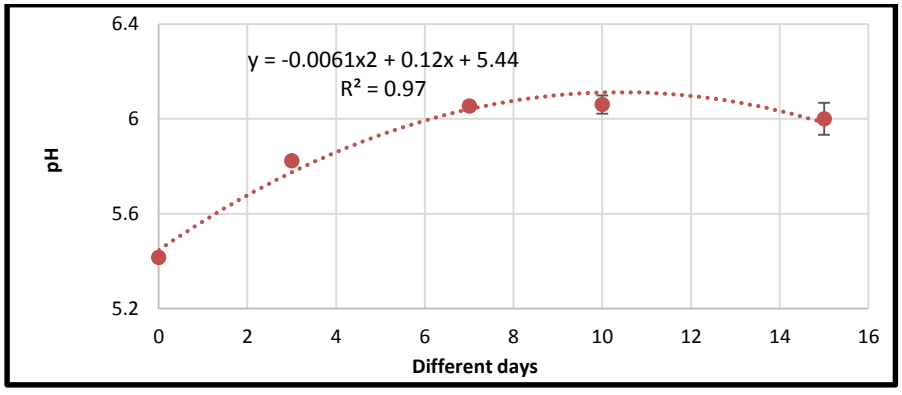

Fig 4-The effect of different storage times on pH (Single interaction)

As you see in figure 5, the results showed that the effects of double interaction between different concentration of gases and layers on pH had significant level (1%).The best result belonged to gas combination (% 30 N2 +% 70 CO2) and 4-layer which pH was observed at its limit, but pH of gas composition 4 in 3-layer (7 µ**)**, was higher than the other gas compositions. However, gas mixtures (% 30 N2 +% 70 CO2) and (% 70 N2 +% 30 CO2) and vacuum packaging could be a great help to control pH of fresh meat ostrich. Container 2 (4-layer) had better effect due to the thickness (131 µ) ,low permeability and water vapor of gas composition (70% CO2 + 30% N2) had the lowest amount of pH due to mechanism could be described by its solution in water of food tissue and produced carbonic acid , which the more carbonic acid was decreased pH.

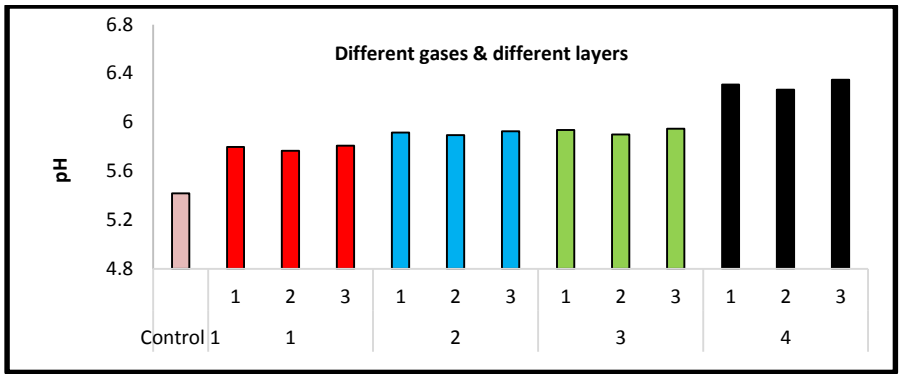

Fig 5-The effect of different concentration of gases & multi layers on pH (Double interaction)

As you see in figure 6,the results showed that the effects of double interaction between different layers over the time on pH during storage times (15 days) had increased slower rate in container 2 (4-layer). The amount of pH in the third - seventh - tenth – fifteenth days were less increased, so could inhibited better than other layers .But pH of samples in 3-layer (AL: 7) increased rapidly ,and had less effect on pH, finally concluded the amount of pH in fresh ostrich meat after 15 day in 3-layer (AL: 7) was highest amount and the lowest pH in this day belonged to 4-layer which had not significant differences with 3-layer (AL:12 µ). The amount of pH were shown that samples had the largest amount after 15 day and the lowest amount after 3 day.

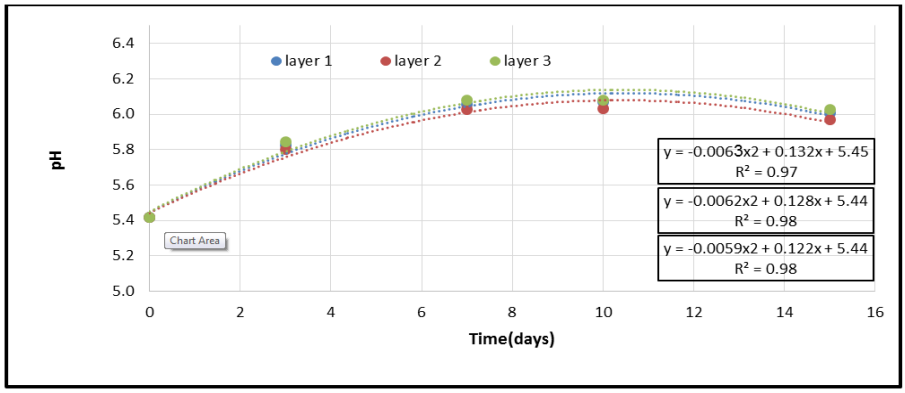

Fig 6-The effect of different storage times & multi layers on pH (Double interaction)

As you see in figure 7,the results showed the effects of double interaction between different gas composition over the time on amount of pH during storage times (15 days) had slower changed in gas composition 1 (% 30 N2 + % 70 CO2) than other gas composition 2 , 3 and 4. And amount of pH in container with gas composition (% 30 N2 + % 70 CO2) after 15 day storage time was acceptable for consumption ,but container with gas compositions 4 , gas composition 3 and also gas composition 2 in day 15 were decreased rapidly, and un acceptable. The gas composition (70% CO2 + 30% N2) had, the lowest pH after 15 day due to mechanism could be described by its solution in water of food tissue and produced carbonic acid which the more carbonic acid decreased pH.

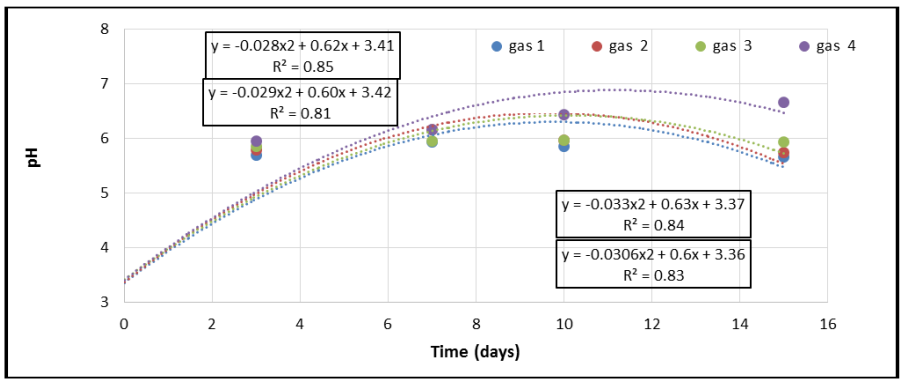

Fig 7-The effect of different concentration of gases & storage times on pH (Double interaction)

As you see in figure 8,the conclusion showed that the triple interaction between different layers ,different composition of gases in different times on pH had changed slower in gas composition 1 (% 30 N2 + % 70 CO2) and 4-layer ,so had the best effect amount of pH. The control sample in 3-layer (AL: 7) have been raised rapidly. Amount of pH in container 1 with gas composition 1 and gas composition 2 from day zero up to day seventh of preservation, had increased and then, has started to decline and this container in gas composition 3 was less severe than gas composition 1 and 2, but in gas composition 4, pH from day zero up to day fifteenth was raised, the best effect related to gas composition 1, gas composition 3 and gas composition 2. Amount of pH in container 3, with gas composition 1, gas composition 2, gas composition 3, as you considered, from day zero up to day seventh of preservation had increased and by passing the day seventh has started to decline, but pH of meat in ordinary condition from day zero up to day fifteenth has increased significantly. Amount of pH of ostrich meat in gas composition 1, gas composition 2 and also gas composition 3 from day zero up to day seventh of preservation has increased. And after passing the day seventh up to the end of the preservation period (the day fifteenth), pH has decreased, which these changes was significant. Therefore pH affected by percentage of CO2 and thickness of container and storage times, have decreased.

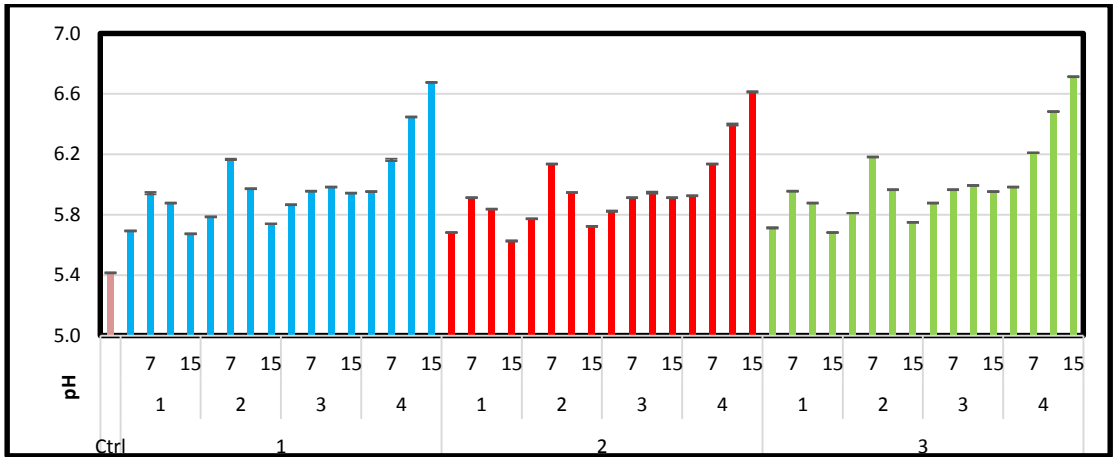

Fig 8- The effect of different concentration of gases, multi layers & storage times on pH (Triple interaction)

DISCUSSION

1- The pH variable had not decreased rapidly, in each treatments during storage times (15 days), while could be explained by characteristic of this multi-layer flexible pouch with less water vapor and oxygen permeability, and the privilege of MAP caused to control chemical reactions such as pH in samples. The lowest amount of pH in packed fresh ostrich meat belonged to layer: 2(4-layer) under gas composition CO2 70%, but maximum change of pH belonged to layer: 3(3-layer) under ordinary condition and then vacuum. Because of the thickness and type of gas atmosphere, which were effected on pH of fresh samples till 15 days.

2- The modified atmosphere packaging (MAP) was not lead to stop spoilage completely .The effect of MAP was not adequate, using this technique controlled pH without a significant adverse effect on food properties. Amount of pH of samples in various conditions, had significant differences between (layer, gas), and (layer, time) and also (gas, time) ($P<0.01$).

Seydim *et al*., 2006, due to research about the shelf life and growth of anaerobic bacteria of packaged ostrich meat under vacuum and MAP condition, the results of pH were corresponded with these results. Chouliara & Karatapanis, 2007, indicated that due to effect of modified atmosphere packaging on shelf-life extension of fresh chicken meat, the results of pH changes, were similar to these results. Taloyor, 2008, conducted due to effect of packaging fresh meat and poultry on pH under, MAP conditions with high amount of CO2 was better than vacuum for pH more than 6, that the results were similar to this study , and the best condition belonged to CO2 70% too. Fernandez-Lopez *etal*., 2008, due to research about effect of packaging conditions on shelf-life of ostrich steaks, the results were corresponded with this investigation. Zand & Allahyari, 2013, due to effect of packaging under gas combination (%30 N2 + %70 CO2) in 4-layer flexible films (131 µ) on shelf life and pH of candy bread was better than 3-layer flexible films, that the results were similar to this investigation. Zand & Sotoudeh, 2013,due to effect of packaging with multilayer flexible pouch on shelf life of chicken meal and changes of pH in 3-layer and 4-layer from day 0 to day 7 of preservation were increased and from day 7 till day 15 were reduced. But the lowest changes of pH belonged to gas combination (%30 N2 + %70 CO2) in 4-layer flexible films (131 µ) and the highest observed in ordinary condition in 3-layer flexible films (119 µ), that the results were corresponded with this investigation. Zand & Sotoudeh, 2013, due to research about the effect of packaging under gas combination (%30 N2 + %70 CO2) in 4-layer flexible films (131 µ) on shelf life and pH of chicken meal, was better than 3-layer flexible films (124 µ) during 20 days that the results were similar to this investigation.

Sotoudeh *et al* , 2013, due to research about usage of MAP for shelf life extension of packed spicy chicken meal in multilayer flexible pouches 4-layer container was better than 3-layer during 20 days and best gas combination belonged to CO2 %70, results of changes of pH were corresponded with these results. Zand, 2013, indicated that due to shelf life extension of mushroom meal in multilayer flexible pouches 4-layer container was better than 3-layer during 60 days, results on changes of pH were corresponded with these results. Zand, 2013, conducted due to shelf life prolongation of packed vegetables meal in multilayer flexible pouches 4-layer container was better than 3-layer during 60 days, results on changes of pH were similar to these results.

CONCLUSION

In the present study, it was concluded that, chemical reaction such as pH in packed fresh ostrich meat have been affected by different flexible multi-layer containers and different concentrations of two gas mixture (carbon dioxide, nitrogen), and also vacuum conditions during 15 days. Our results confirmed, the modified atmosphere packaging (MAP) was not lead to stop spoilage completely but delayed it .The effect of MAP was not adequate but using this technique inactivated microorganism without a significant adverse effect on food properties and also controlled pH of ostrich meat samples. These parameters could be promoted, substitution of these barrier pouches and MAP instead of traditional packaging in meat packaging, due to a lot of privilege of them for shelf life extension of ostrich meat in long times.

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