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Effect of MAP and Multi-layer Flexible Films on the Growth of Aerobic Bacteria of Fresh Ostrich Meat

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ABSTRACT

The effect of different concentrations of two gas mixture (Carbon dioxide, Nitrogen), and also vacuum conditions and the usage of flexible multi-layer films has been studied on the growth of aerobic bacteria in fresh meat ostrich at refrigerator (T=4 ^{0}C). Ordinary conditions as a control packaging were compared with three types of modified atmosphere packaging: {(N270% + CO230%), (N230% + CO270%)} and vacuum conditions in this study. Fresh ostrich meat were packaged into 3 kinds of polymeric flexible pouch" 4-layers {PET (12) / AL (7) / PET (12) / LLD (100)}, 3-layers {PET (12) / L (12) / LLD (100)} and 3-layers {PET (12) / AL (7) / LLD (100)}. Samples were performed microbial tests (Total count of aerobic bacteria), in 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 usage of MAP was not adequate for controlling spoilage, but the spoilage process was delayed. Aerobic bacteria's grew in fresh ostrich meat, and their population under (% 30 CO2 +% 70 N2) and also vacuum conditions in these containers were more than 10^6 (not acceptable).However, the best condition belonged to (N2 30% N2+ 70% CO2), which was acceptable. The Characteristic of these multi-layer flexible pouches (3-4 layers) with less water vapor and oxygen permeability, caused to control some chemical reactions and microbial growth in samples. Maximum aerobic bacteria's which were grown in these meat samples was related to control conditions, then treatments under vacuum and % 30 CO2 in 3-layers AL (7 μ), and the lowest growth belonged to treatment under % 70 CO2 in 4-layer because of thickness and low steam permeability of this 4-layer container and anti-bacterial properties of more percentage of CO2.

Keywords: modified atmosphere packaging (MAP), aerobic bacteria growth, fresh ostrich meat, flexible multi-layer films.

INTRODUCTION

Ostrich meat due to low fat meat (saturated fat), the lowest cholesterol levels, too much iron is a good source of vitamins A, B_1, B_6, B_{12} can be substituted as new red meat instead of other presented meats [1,3,11]. The smell and taste of fresh ostrich meat are similar to red meats such as veal and sheep meats according to consumer tastes [1, 14]. Fresh ostrich meat is usually sold before 24-48 hours after death, and must be cold immediately. Packaging of fresh and frozen ostrich meat (crushed and minced) are needed for shelf life prolongation [1,11,14]. The modified atmosphere packaging (MAP) is well known for various researchers which there is a non-thermal method for inactivation microorganism and is widely used for shelf-life extension and improvement the quality of perishable foods stored at refrigeration temperatures [16,29-32]. However there is no degradation of flavor and taste with heat denaturation of objectives [12]. The ability of modified-atmosphere packaging for extending the shelf life of foods has been recognized for many years. Indeed, over 100 years ago [2,4,5]. Modified atmosphere packaging is the enclosure of a food, in a package which the atmosphere has been changed by altering the proportions of carbon dioxide, oxygen, nitrogen, water vapor and trace gases. The process limits microorganism as well as biochemical activity. This modification is performed by gas flash packaging which air is removed and replaced by a controlled mixture of gases [5,16,29-32]. MAP inhibits some microorganisms, so can increase the quality of variety foods. These products (fresh ostrich meat) without an efficient processing are potential source of pathogenic

microorganisms, since the low acidity (pH 5-5.5) and suitable water activity of these packed meats, create an ideal environment for rapid microbial spoilage in these packages [10,13]. Although, thermal treatment (120 ^{o}C, 20 min) effectively destroys these microorganisms [6,16,29-32], has been used widely, proteins and some other physiological substrates are inactivated, and consequently the sensory properties and contents of nutrients in foods are lost [5,16,29-32]. For that reason, significant efforts are leading to the development of novel processing such as MAP [5,7,16,29-32], which is proving to be able to inactivate spoilage microorganisms without significantly affect nutritional and sensory properties of several foods [10,12]. However the growth of microorganisms depends on temperature, pH and water activity as the main growth-determining factors, other factors can significantly influence the growth characteristics of the microorganism. 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 [7,17,29-32], 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 (5,7,16). Nitrogen (N2) is a non-reactive gas that has no smell or taste, unlike carbon dioxide, is not absorbed in food or water [15,16,29-32]. It is used as a filler gas to replace oxygen and thus prevent spoilage or to replace carbon dioxide and prevent package collapse [29-32]. Other hand the multi-layer films have been used for packaging fresh ostrich meat are plastic films laminated with aluminum [19-28] .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 [27,28]. 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 [19,20,27]. In this study, we investigate about the effects of modified atmosphere packaging with different concentrations of CO2/N2 and vacuum on growth of aerobic bacteria [16,29-32], and the usage of 3 multilayer flexible pouches [15,16,29-32] for packaging ostrich fresh meat during storage times (15 days). We try to prove MAP can substitute thermal processing in conservation industries, and control growth of aerobic bacteria [19,20,16, 28-32].

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=20 ° C). Samples were ready for packaging. Pouches contained 100 g, fresh ostrich meat [20,22,23, 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 [8,9,20-23, 29-32].

Modified Atmosphere Packaging

Henkelman packing machine, model Boxer-200A was used in this project. Samples were packed into three multilayer flexible pouches (3 and 4 layers) under modified atmosphere. After packaging, samples were put in refrigerator immediately, for evaluation second contamination and growth of aerobic bacteria during 15 days [17,29-32].

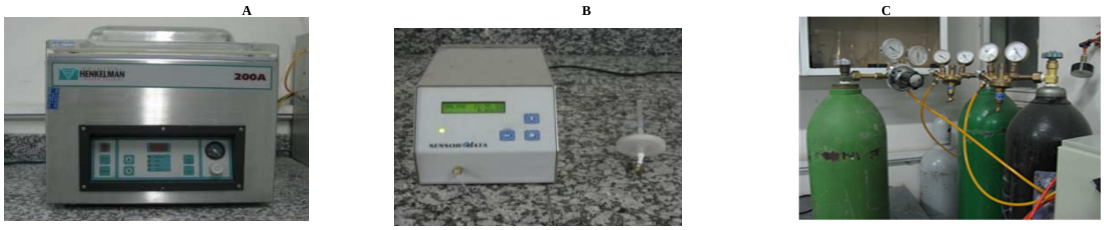

Fig 1.(A) Modified atmosphere packaging, (B) gas analyzer, (C) gas flash tank(Model: Boxer-200A)
[17,29-32]

Microbial culture

Total count of aerobic bacteria in PCA & CMM media

PCA(Peptone from casein 5g/1000 ml; glucose 1g/1000 ml, Yeast Extract 2.5 g/1000 ml, Agar 14g/1000 ml, Distillated water 1000 ml),Plate Count Agar is a general media for aerobic bacteria count. **CMM** (Beef heart 454g/1000 ,Protease peptone 20 g/1000 ml, glucose 5 g/1000 ml, Sodium chloride 5g/1000 ml, Sodium hydrochloride ½ 454 g/1000, Distillated water 1000ml),Cooked Meat Media is an enrichment media for aerobic bacteria [8,9,20-23, 29-32].

First 1-2 g samples was put in CMM (3-4day) while 1 g of enriched sample was weighed under the microbial laboratory hood, and was crushed in 10 ml of ringer's solution. According to CFU method, divided into one series tube (six tubes) which contain 9 cc sterile distilled water. First 1 cc of the sample added to tube no one and transferred tube by tube, main sample was prepared by serial dilution (0.01,0.001…). Finally pour plate method were done in the PCA culture ,too in order to count aerobic bacteria which was incubated for 3 days at 37 ° C [8,9,20-23, 29-32].

Samples packaging and storage

All pouches (unprocessed and processed samples), were put at refrigerator temperature (T= 4° C). Analytical characteristics of these barrier containers were shown in table 1 [17,29-32].

Table 1- Analytical characteristics of containers [16,29-32]

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 growth of aerobic bacteria. Statistical analysis of data , was performed by software Statistical Analysis System (SAS: 9/1) with Anowa test, and comparison of data was performed by Duncan's new multiple range test, with confidence level of 95% ($P < 0.05$) (17,29-32).

RESULTS

Total count of aerobic microorganisms in different conditions

According to analysis of variance table 1, the effect of different layers, gas compositions, times and the double interactions (layers , gases), (layers ,times) and (gases , times) and the triple interactions (layers, gases, times) on aerobics bacteria count were significant level ($p< 0.01$). The primary effects of layers, gas compositions and times on aerobic bacteria 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 (1.448), 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 count of aerobic bacteria
Container	2	**37.531
Gas Composition	3	**37.023
Container * Gas Composition	6	**0.46
Time	3	**47.553
Container * Time	6	**0.47
Gas composition * Time	9	**0.385
Container * Gas Composition * Time*	18	0.495
Error	98	0.005
Coefficient of Variation (CV)	-	1.448

***, * 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 total count of aerobic bacteria in PCA media, the lowest growth of these bacteria were observed in control packaging 1 in initial day. Although the most growth of bacteria were observed in control condition , after 15 days ,and then in vacuum and 30% CO2 condition, as you see in figure 2 .The lowest growth of bacteria belonged to condition under 70% CO2+30% N2 as an anti-bacterial effect.

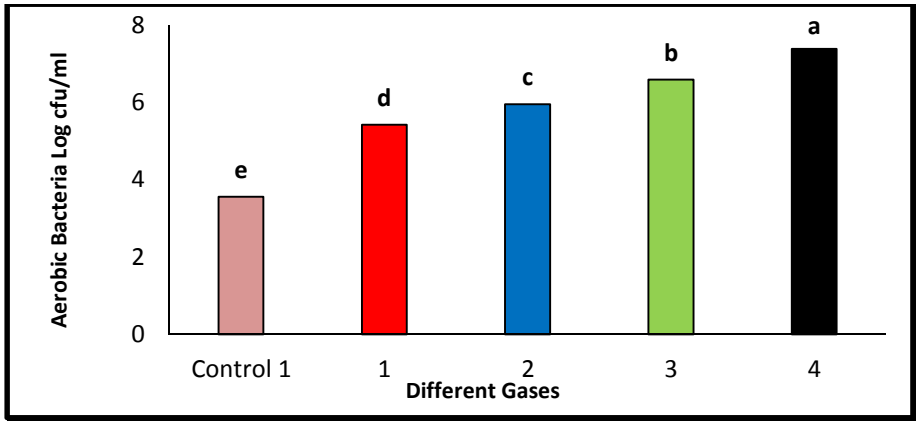

Fig 2-The effect of different concentration of gases on aerobic bacteria(log cfu/ml) (Single interaction)
{1:CO2 70% + N2 30%;2:CO2 30% + N2 70%;3:Vaccum; 4:Control}

According to total count of aerobic bacteria in PCA media, the lowest growth of these bacteria were observed in control packaging 1 as an initial control (glassy container). Howere the most growth of bacteria were observed in 3-layer (AL:7 µ), and then in 3-layer (AL:12 µ), as you see in figure 3 .The best condition belonged to 4-layers due to low steam permeability.

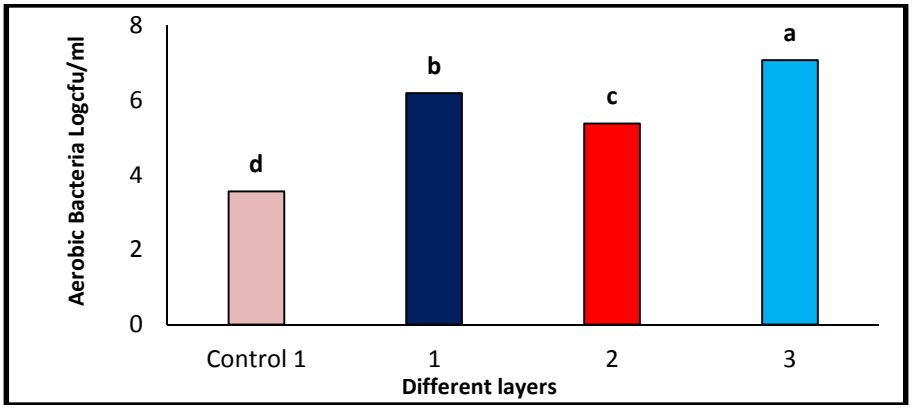

Fig 3-The effect of different layers on aerobic bacteria(log cfu/ml) (Single interaction)
{1: 3- layer (AL:12 µ);2:4-layer ;3: 3- layer (AL:7 µ); 4:Control 1}

As you see in figure 4, total count of aerobic bacteria in PCA media were reported, the lowest growth of these bacteria's in initial day as a control 1. But the most growth of bacteria were observed, after 15 days. This model (y = 0.274x + 3.88) was described the relation between time and growth of microorganism which had significantly level.

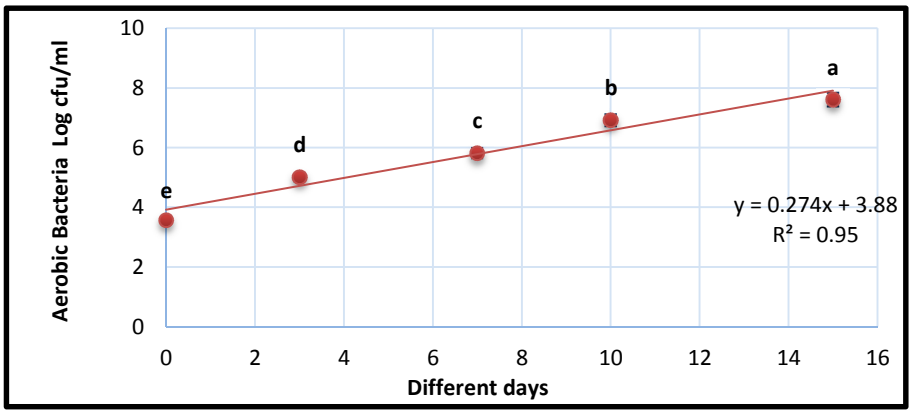

Fig 4-The effect of different storage times on aerobic bacteria(log cfu/ml) (Single interaction)

As you see in figure 5, the results showed that the effects of double interaction between different concentration of gases and layers on total count of aerobic bacteria had significant level (1%).The best result belonged to combination (% 30 N2 +% 70 CO2) and 4-layer which the total count was observed at its limit, but the effect of gas

composition 4 in 3- layer(7 µ**)**, was higher than the other gas compositions and was not acceptable for this study. However, the composition of the gas mixtures (% 30 N2 +% 70 CO2) and (% 70 N2 +% 30 CO2) and vacuum packaging could be a great help to increase the shelf life of fresh meat ostrich. Container 2 (4-layer) had better effect due to the thickness (131 µ) low permeability of water vapor and gas composition (70% CO2 + 30% N2) have the lowest number of bacteria due to the antibacterial properties of carbon dioxide gas**.**

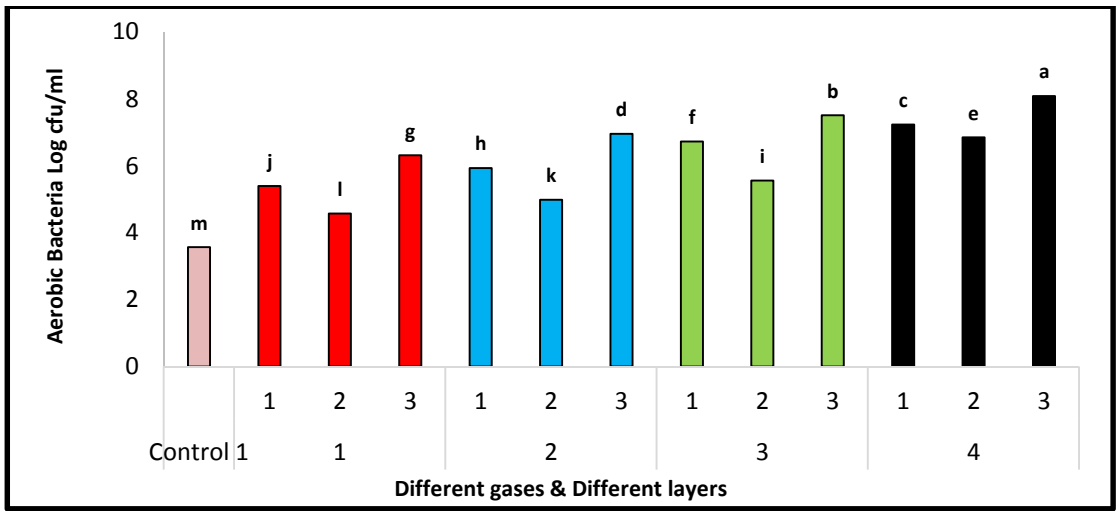

Fig 5-The effect of different concentration of gases & multi layers on aerobic bacteria(log cfu/ml) (Double interaction)

As you see in figure 6,the results showed that the effects of double interaction between different layers over the time on total count of aerobic bacteria during storage times (15 days) had increased slower rate in container 2 (4-layers). The total number of aerobic bacteria in the third - seventh - tenth – fifteenth days were less increased, so could inhibited better than other layers .But aerobic bacteria of samples in 3-layers (AL: 7) increased rapidly ,and had less effect on growth of aerobic bacteria, finally concluded the number of aerobic bacteria in fresh ostrich meat after 15 day in 3-layers (AL: 7) was highest and the lowest total count of aerobic bacteria in this day belonged to 4-layers which was acceptable for this study. Total counts were shown that samples had the largest amount after 15 day and the lowest amount after 3 day, which caused bacteria to grow and multiply rapidly by the times.

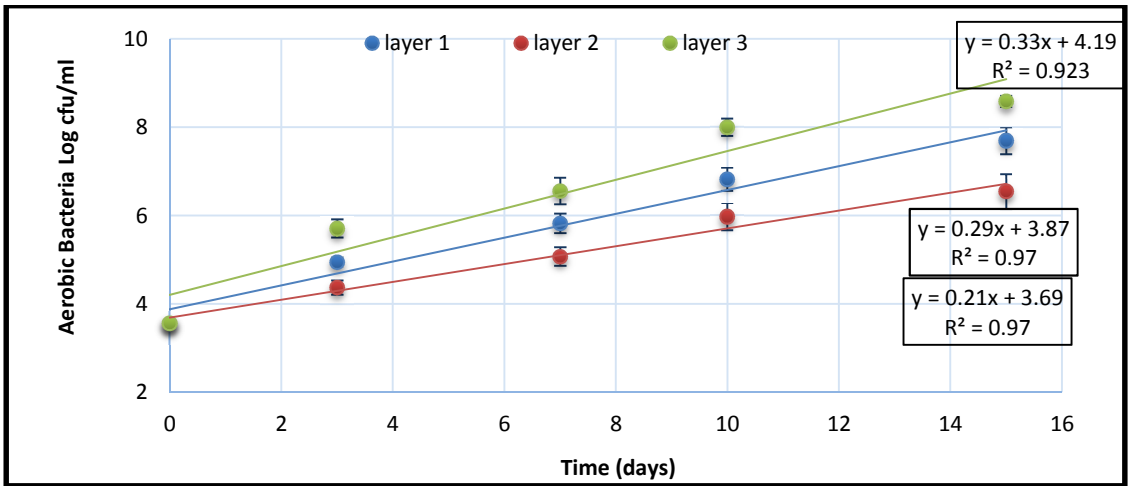

Fig 6-The effect of different storage times & multi layers on aerobic bacteria(log cfu/ml) (Double interaction)

As you see in figure 7,the results showed the effects of double interaction between different gas composition over the time on total count of aerobic bacteria during storage times (15 days) had increased slower in gas composition 1 (% 30 N2 + % 70 CO2) than other gas composition 2 and 3 and 4. And total count of aerobic bacteria in container with gas composition (% 30 N2 + % 70 CO2) after 15 day storage time was not increased rapidly so was acceptable for consumption ,but container with gas compositions 4 and gas composition 3 in day 15 were increased

rapidly ,and un countable . The gas composition 70% CO2 + 30% N2 had, the lowest number of bacteria after 15 day due to the antibacterial properties of carbon dioxide gas**.**

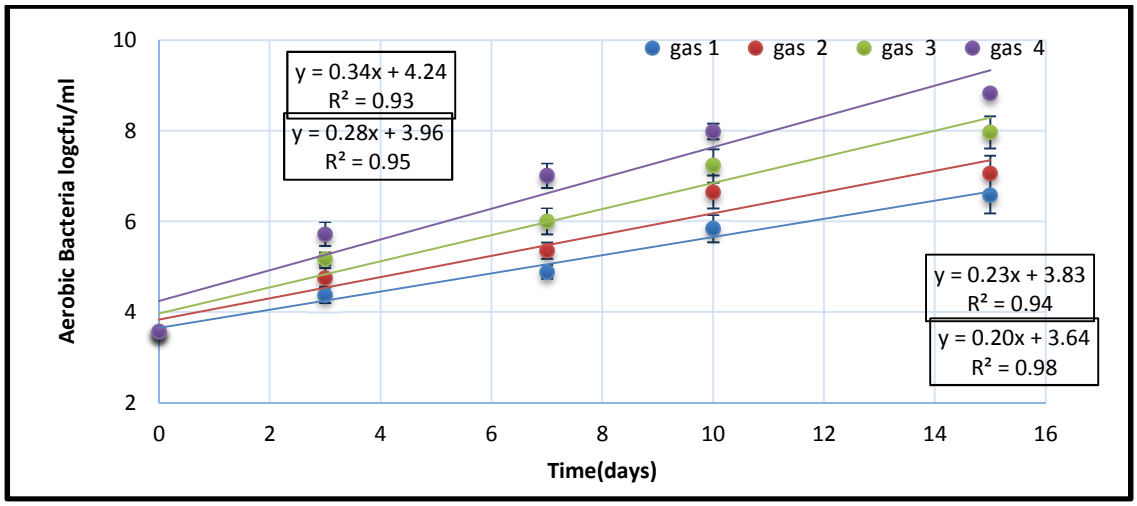

Fig 7-The effect of different concentration of gases & storage times on aerobic bacteria(log cfu/ml) (Double interaction)

As you see in figure 8,the conclusion showed that the triple interaction between different layers ,different composition of gases in different time on total count of aerobic bacteria had increased slower in gas composition 1 (% 30 N2 + % 70 CO2) and 4-layer, so had the best effect on the total count of aerobic bacteria , over the time, number of aerobic bacteria were in limit (the 10^6) and was not increased , then 4-layers and composition of gas 2 (% 70 N2 +% 30 CO2) and 4-layers and vacuum had been effective so the shelf life had evaluated more than 10 days (according to amount of aerobic bacteria). The control sample in all three containers have been kept only 3-4 day, which the total number of aerobic bacteria was not acceptable for consumption (un countable). Samples were packed in 3 layer (AL: 12) was acceptable after 7 day of storage. The use of container with greater thickness and less permeability with the composition of gas (higher amount of CO2), gas composition 1 (% 30 N2 + % 70 CO2) is more effective and had the best inhibitory effect on growth of aerobic bacteria. As the above results, were shown, the total number of aerobic bacteria s affected by antibacterial effects of percentage of CO2 and thickness of container, have decreased.

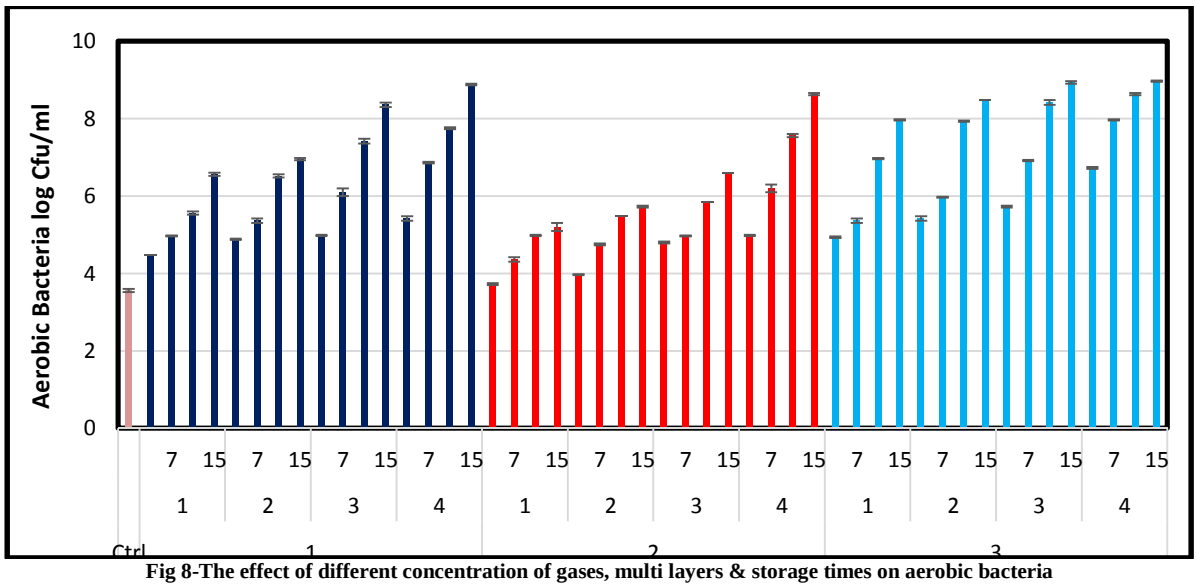

Fig 8-The effect of different concentration of gases, multi layers & storage times on aerobic bacteria (log cfu/ml) (Triple interaction)

DISCUSSION

The results of this study showed that CO2 had antimicrobial effect and its mechanism could be described by its solution in water of food tissue and produced carbonic acid, which the carbonic acid arrived to cell membrane of

microorganisms and ionized into the cell and the collapsed electrical balance within the cell in order to killing microorganisms . The difference between microbial activities in the samples was significantly dependent on the concentration of nitrogen and carbon dioxide, as well as the role of nitrogen gas, indirectly could influenced perishable foods by decreasing the growth of aerobic micro-organisms. The second role of nitrogen in modified atmosphere packaging, was a gas filler and protecting the development of flexible packaging against vacuum.

1- The lowest amount of aerobic bacteria in packed fresh ostrich meat belonged to layer:2 (4-layers) under gas composition CO2 70%, but highest amount belonged to layer :3(3-layers) under ordinary condition and then vacuum. Because of the thickness and of type gas atmosphere, which were affected on growth of aerobic bacteria of fresh samples till 15 days.

2-Aerobic bacteria count of samples in various conditions, had significant differences between (layers, gas), and (layer , time) and also (gas , time) ($P<0.01$).

Stier *et. al.,* 1981, due to research about amount of aerobic and anaerobic microorganism in packed salmon fillet, the results of aerobic count were corresponded with these results and reliable. Grua, 1983, due to research about microbial growth in different packed meat using multilayer film in 0ºc, under vacuum condition, the results of aerobic bacteria count were corresponded with this investigation.

Ozbas *et al*.,1997, due to effects of modified atmosphere and vacuum packaging on spoilage and inoculated pathogenic bacteria on fresh poultry, the results of aerobic count were corresponded with these results. Gill, 2000 , due research about effect of modified atmosphere and vacuum packaging on the growth of pathogenic bacteria on fresh poultry, the results of aerobic bacteria count were similar to these results.

Vanderzant *et al*., 2000 indicated that due to the shelf life growth of aerobic bacteria of beef steak packaged under vacuum and MAP condition in different barrier containers during 30 days were significant difference with ordinary condition as a control and the results were corresponded with these results. Chouliara & Karatapanis, 2007, due to combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat , the results of aerobic bacteria count were similar to these results .Bingol & Ergun, 2011 , indicated that due to effect of two different types gas carbon dioxide and oxygen had been perform on ostrich meat, the results showed that the shelf life of ostrich meat % 60 CO2 prolonged till 7 day, were corresponded with these results. Sotoudeh *et al* , 2013, concluded 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, best condition belonged to CO2 %70, results were corresponded to these results. Zand & Sotoudeh, 2013,due to effect of packaging under gas combination(%30 N2 + %70 Co2) in 4-layer flexible films (131 µ) on shelf life and aerobic bacteria of chicken meal, was better than 3-layer flexible films (124 µ) during 20 days that the results were similar to this investigation. Zand & Allahyari, 2013, due to research about effect of packaging under gas combination (%30 N2 + %70 Co2) in 4-layers flexible films (131 µ) on shelf life and aerobic bacteria of candy bread ,was better than 3-layer flexible films (124 µ) during 20 days that the results were similar to this investigation. Zand, 2013, indicated that due to shelf life extension of mashroom meal in multilayer flexible pouches 4-layer container was better than 3-layer during 60 days, results were corresponded with these results . Zand, 2013, concluded due to the shelf life prolongation of packed vegetables meal in multilayer flexible pouches 4-layer container was better than 3-layer during 60 days, results were corresponded with these results.

CONCLUSION

In the present study, it was concluded that, shelf life and growth of aerobic bacteria of packed ostrich meat have been affected by different flexible multi-layer pouches 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 postponed it .The effect of MAP was not adequate but using this technique inactivated aerobic microorganism without a significant adverse effect on food properties of ostrich meat samples. These parameters could be promoted substitution of MAP and these barrier containers instead of other traditional meat packaging, due to a lot of privilege for shelf life extension of meat in long times

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