



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

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### ABSTRACT

The usage of different concentrations of two gas mixture (Carbon dioxide, Nitrogen), and also vacuum conditions and the effect of flexible multi-layer pouches has been studied on the growth of anaerobic bacteria in fresh ostrich meat at refrigerator ( $T=4^{\circ}\text{C}$ ). Ordinary condition as a control packaging was compared with three types of modified atmosphere packaging:  $\{(\text{N}270\% + \text{CO}230\%), (\text{N}230\% + \text{CO}270\%)\}$  and vacuum conditions in this project. Ostrich fresh meat were packaged into 3 kinds of polymeric flexible pouch" 4-layers with thickness  $131\mu$ ,  $\{\text{PET} (12) / \text{AL} (7) / \text{PET} (12) / \text{LLD} (100)\}$ , 3-layer with thickness  $124\mu$   $\{\text{PET} (12) / \text{AL} (12) / \text{LLD} (100)\}$  and 3-layer with thickness  $119\mu$   $\{\text{PET} (12) / \text{AL} (7) / \text{LLD} (100)\}$ . Samples were performed microbial tests (Total count of anaerobic bacteria), in different times 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. Anaerobic bacteria population of ostrich meat under ( $\% 30 \text{ CO}_2 + \% 70 \text{ N}_2$ ) and also vacuum conditions in this container were more than  $10^4$  (not acceptable). However, the best condition belonged to ( $\text{N}_2 30\% + \text{CO}_2 70\%$ ), and was acceptable till 15 days. Maximum anaerobic bacteria s which were grown in these meat samples was related to  $\% 30 \text{ CO}_2$  conditions in 3-layer Al( $7\mu$ ), and the lowest growth belonged to treatment under  $\% 70 \text{ CO}_2$  in 4-layer. The Characteristic of this multi-layer flexible pouch (4-layers) with less water vapor and oxygen permeability and also increasing more percentage of  $\text{CO}_2$  due to antibacterial properties of carbon dioxide gas, caused to control microbial growth in samples, so could be extended shelf life of fresh ostrich meat.

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

### INTRODUCTION

The modified atmosphere packaging (MAP) is well known that 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 [2,4,15,29-32]. However there is no degradation of flavor and taste with heat denaturation of objectives [11]. The ability of modified-atmosphere packaging for prolonging the shelf life of foods has been known for many years [3,5,16]. 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 [2,9,15,29-32]. 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 this package meals [9,12]. Although, thermal treatment ( $120^{\circ}\text{C}$ , 20 min) effectively destroys these microorganisms [15,29-32], 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 [15,17,29-32]. For that reason, significant efforts are leading to the development of novel processing such as MAP [2,16,17, 29-32], which is proving to be able to

inactivate spoilage microorganisms without significantly affect nutritional and sensory properties of several foods [9,11] 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 CO<sub>2</sub>/ N<sub>2</sub> concentration (%) in the head space of pouches as the independent variable for the gas atmosphere demonstrated that CO<sub>2</sub> exerts as an antimicrobial effect in the water-phase of the food product [8,15,29-32], therefore except the effect of intrinsic, extrinsic and processing parameters on the CO<sub>2</sub> solubility, the concentration of dissolved CO<sub>2</sub> in the water-phase of the food products should be incorporated in this study as independent variable [4,5,15]. Nitrogen (N<sub>2</sub>) is a non-reactive gas that has no smell or taste, unlike carbon dioxide, is not absorbed in food or water [14,15,29-32]. 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]. Sensory analysis is unequivocally assigning in the scientific methods. It is one of the oldest means of quality control, but in principle is an essential part of the mandatory assessment of food quality, while also examining the deeper study of the interdependence between physiological and psychological phenomena in the very process of perception of sensory qualities [30-32]. Ostrich meat due to low fat meat (saturated fat), the lowest cholesterol levels, too much iron is a good source of vitamins A, B<sub>1</sub>, B<sub>6</sub>, B<sub>12</sub> can be substituted as a new red meat instead of other presented meats [1,10,13]. Red meat such as beef and sheep meat on the floor, with a taste close to veal and beef are in accordance with consumer tastes. Fresh Ostrich meat is usually sold before 24-48 hours after death are cold and immediately vacuum packed [1,10]. Packaging of fresh and frozen ostrich meat (crushed and minced) are needed for shelf life prolongation [1, 10]. Other hand the multi-layer films have been used for packaging these meat are plastic films laminated with aluminum for packaging cooked meat and cooked poultry instead of can [18-28]. These laminated packages with some metal component can considerably change the food temperatures and also microwave transparent with a high melting point [18-21,27,28]. 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 [18,19,20,27]. In this study, we evaluate the effects of modified atmosphere packaging; different concentrations of CO<sub>2</sub>/N<sub>2</sub> growth of anaerobic bacteria [17, 29-32], and the usage of 3 types of multilayer flexible pouch and MAP [17,29-32] of fresh ostrich meat during storage times (15 days) . We want to promote MAP can substitute thermal processing in conservation industries, and control growth of anaerobic bacteria [18-20, 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. The samples were washed, fat were isolated and then samples were cut into small pieces (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 [20,21,23 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 [6,7,20-23, 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-layer (PET<sub>(12)</sub>/AL<sub>(12)</sub>/LLD<sub>(100)</sub>), and 4-layer (PET<sub>(12)</sub>/AL<sub>(7)</sub>/PET<sub>(12)</sub>/LLD<sub>(100)</sub>), and 3-layer (PET<sub>(12)</sub>/AL<sub>(7)</sub>/LLD<sub>(100)</sub>) under modified atmosphere (16,17,21). After packaging, samples were put in at refrigerator (T= 4 °C) immediately, for evaluation second contamination and growth of anaerobic bacteria [15,29-32].



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

### Microbial culture

#### Total count of anaerobic bacteria in PE 2 & BHI culture

**BHI** (Beef heart infusion solids 17.5 g/litre, Proteose peptone 10.0 g/litre, Glucose 2.0 g/litre, Sodium chloride 5.0 g/litre, Di-sodium phosphate 2.5 g/litre, pH 7.4 ± 0.2 @25°C). BHI is a general media for anaerobic bacteria count. **PE 2** (Peptone digest of animal extract 20 g/1000 ml, Yeast Extract 3g/1000 ml, 2% Alcoholic solution of

bromocresol purple 0.04g/1000 ml, Cicer arietinum L450 no, Distillated water 1000 ml) Peptone Yeast Extract bromocresol Purple is an enrichment media for anaerobic bacteria [6,7,20-23, 29-32].

First, 1 g sample under the hood of microorganisms in the laboratory was weighed and was crushed in 10 ml Ringer's solution, then PE2 (10 ml) was added which was a culture for enrichment and with gas pack type A in anaerobic jar, for 3 days was incubated at 37 °C, 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 double BHI culture ,too in order to count anaerobic bacteria which was incubated for 4 days at 37 °C [6,7,20-23, 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 [15,29-32].

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 <sup>2</sup> .day)	W.V.T.R (g/ m <sup>2</sup> .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 anaerobic bacteria. Statistical analysis of data, was performed by software Statistical Analysis System (SAS: 9/1) with Anova test, and comparison of data was performed by Duncan's new multiple range test, with confidence level of 95% (P <0.05) [15,29-32].

## RESULTS

### Total count of anaerobic bacteria 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 anaerobic bacteria were significant (p< 0/01) . The primary effects of layers, gas compositions and times on anaerobic 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 2 -3 (2.737), 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 anaerobic bacteria
Container	2	**13.213
Gas Composition	3	**5.11
Container * Gas Composition	6	**0.55
Time	3	**4.884
Container * Time	6	**0.079
Gas composition * Time	9	**0.234
Container * Gas Composition * Time*	18	0.07
Error	98	0.007
Coefficient of Variation (CV)	-	2.737

- \*\*, \* and <sup>ns</sup>, significance at 1% and 5% and non-significance, respectively.

Table 3-Different concentration of 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	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 anaerobic bacteria in BHI media, the lowest growth of these bacteria were observed in control sample in initial control as a glassy container. Howere the most growth of bacteria were observed in 3-layer (AL:7 μ) , after 15 days and , then in 3- layer (AL:12 μ),which had not significant level ,as you see in figure 2 .The best condition belonged to 4-layers and was significant level in comparison with other containers due to less permeability.

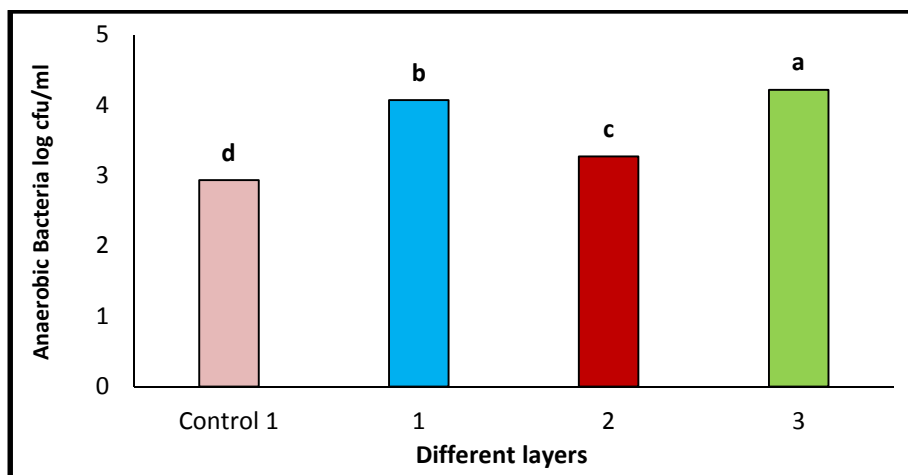


Fig 2-The effect of different layers on anaerobic bacteria (log cfu/ml) (Single interaction)  
{1: 3- layer (AL:12  $\mu$ ); 2:4-layer; 3: 3- layer (AL:7  $\mu$ ); 4:Control 1}.

According to total count of anaerobic bacteria in BHI media, the lowest growth of these bacteria were observed in control sample as an initial control. Although the most growth of bacteria were observed in condition under ( 30% CO<sub>2</sub>+70% N<sub>2</sub>), and then in vacuum condition, as you see in figure 3. The lowest growth of bacteria belonged to ordinary condition as a control, and ( 70% CO<sub>2</sub>+30% N<sub>2</sub>) too, which had not significant level due to anti-microbial properties.

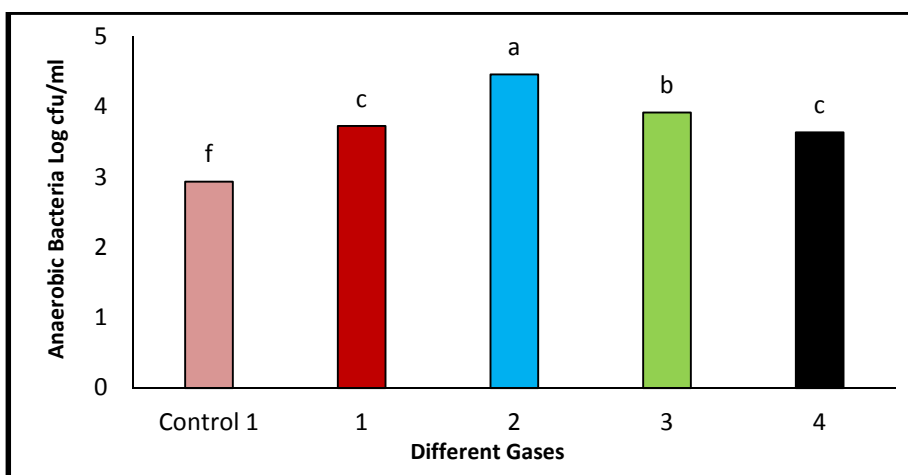


Fig 3-The effect of different concentration of gases on anaerobic bacteria (log cfu/ml) (Single interaction)  
{1:CO<sub>2</sub> 70% + N<sub>2</sub> 30%;2:CO<sub>2</sub> 30% + N<sub>2</sub> 70%;3:Vacuum; 4:Control}.

As you see in figure 4, total count of anaerobic bacteria s in BHI media were reported, the lowest growth of these bacteria in initial day as a control 1. But the most growth of bacteria were observed, after 15 days. This model ( $y = 0.341x + 2.282$ ) was described the relation between time and growth of microorganism which had significantly level.

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 anaerobic bacteria had significant level (1%). The best result belonged to control and combination ( % 30 N<sub>2</sub> + % 70 CO<sub>2</sub>) in 4-layer which the total count was observed at its limit, but the effect of gas composition 2 ( % 70 N<sub>2</sub> + % 30 CO<sub>2</sub>) in 3-layer (7  $\mu$ ), was higher than the other gas compositions and was not acceptable for this study. However, the composition of the gas composition 1 ( % 30 N<sub>2</sub> + % 70 CO<sub>2</sub>) and control and also vacuum condition could be a great help to decrease anaerobic bacteria of fresh meat ostrich. Container 2 (4-layer) had better effect due to the thickness (131  $\mu$ ) low permeability of water vapor and gas composition 1 after initial control condition have the lowest number of bacteria (similar to control) due to the antibacterial properties of carbon dioxide gas.

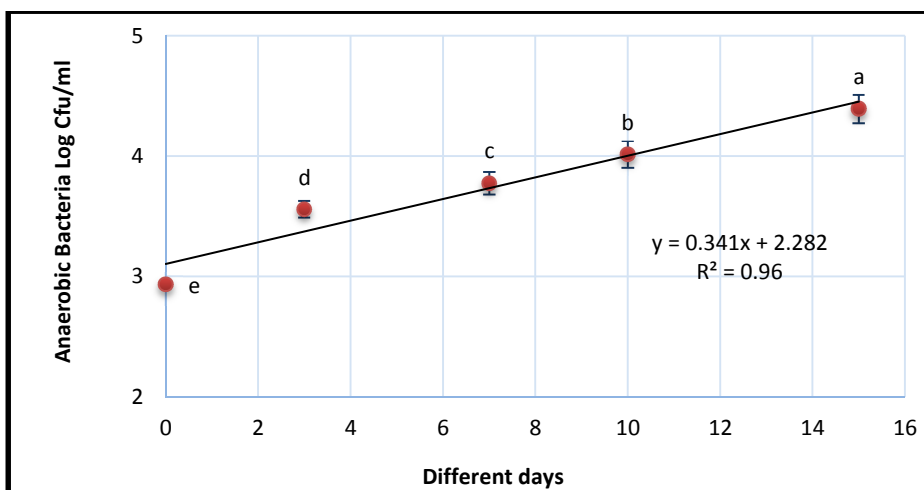


Fig 4-The effect of different storage times on anaerobic bacteria( log cfu/ml) (Single interaction)

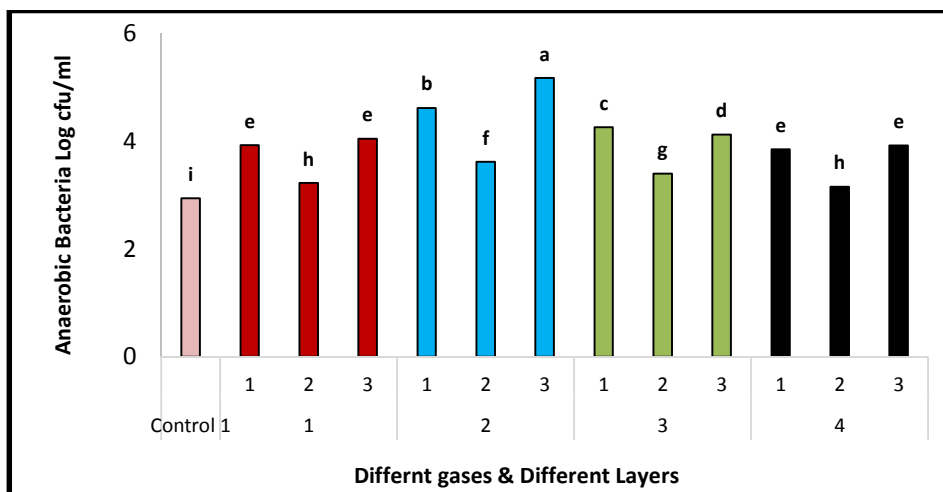


Fig 5-The effect of different concentration of gases & multi layers on anaerobic bacteria( log cfu/ml) (Double interaction)

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 anaerobic bacteria during storage times (15 days) had increased slower rate in container 2 (4-layers). The total number of anaerobic bacteria in the third - seventh - tenth - fifteenth days were less increased, so could inhibited better than other layers. But anaerobic bacteria of samples in 3-layers (AL: 7) increased rapidly, and had less effect on growth of anaerobic bacteria, finally concluded the number of anaerobic bacteria in fresh ostrich meat after 15 day in 3-layers (AL: 7) was highest and the lowest total count of anaerobic 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.

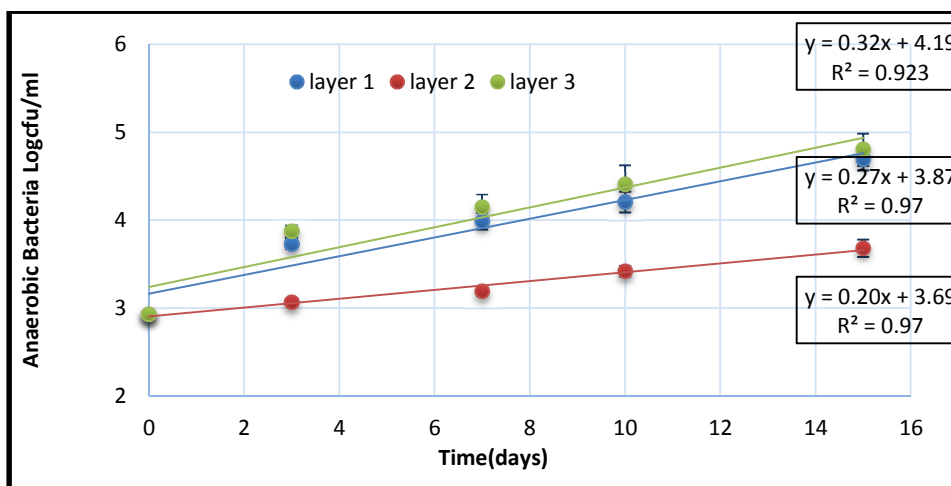


Fig 6-The effect of different storage times & multi layers on anaerobic bacteria( log cfu/ml) (Double interaction)

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 anaerobic bacteria during storage times (15 days) had increased slower in gas composition 1 (% 30 N2 + % 70 CO2) after initial control condition than other gas composition 2 and 3. And total count of anaerobic bacteria in container with gas composition (% 30 N2 + % 70 CO2) and ordinary condition after 15 day storage time was not increased rapidly so was acceptable for consumption ,but container with gas compositions 2 and gas composition 3 in day 15 were increased rapidly ,and un countable . The gas composition (70% CO2 + 30% N2) after ordinary treatment had, the lowest number of an anaerobic bacteria after 15 day due to the antibacterial properties of carbon dioxide gas.

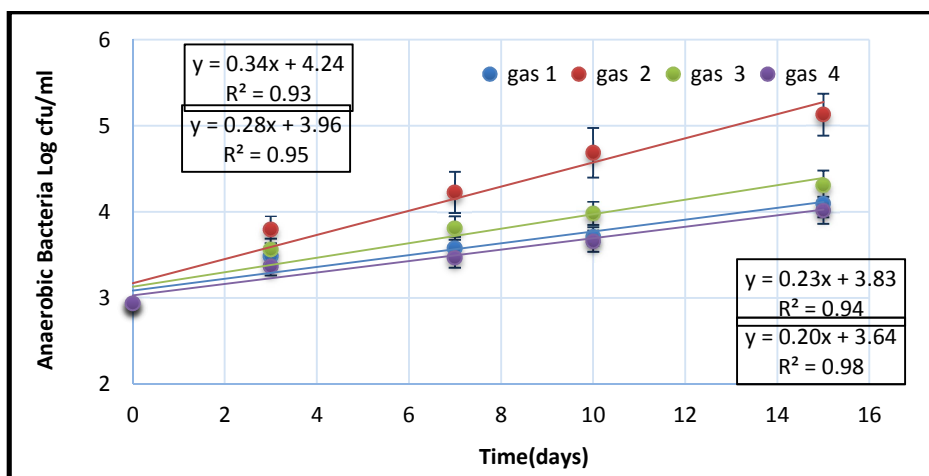


Fig 7-The effect of different concentration of gases & storage times on anaerobic bacteria( log cfu/ml) (Double interaction)

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 anaerobic bacteria had increased slower in gas composition 1 (% 30 N2 + % 70 CO2) after ordinary condition in 4-layer ,so had the best effect on the total count of anaerobic bacteria , over the storage time, which number of anaerobic bacteria were in limit (the 10<sup>4</sup>) and was not increased , then 4- layers under composition of gas 3 (vacuum) had been effective. The sample under gas composition 2 (% 70 N2 + % 30 CO2) in container 3 after 15 days, was not acceptable (un countable). The effect of container 2 during storage times after 3,7,10,15 days, on the growth of anaerobic bacteria under mixture of gas 4 gas composition 1 gas composition 3 had not a lot of significant difference. The overall growth of anaerobic bacteria in the container 3 during storage times after 3,7,10,15 days ,under gas composition 1 and gas composition 4 there had not significant difference, and had the lowest number of these bacteria, then gas composition 3 (vacuum) in container 3 was reported. 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) after control condition was more effective and had the best inhibitory effect on growth of anaerobic bacteria. As the above results, were shown, the total number of

anaerobic bacteria s affected by antibacterial effects of percentage of CO<sub>2</sub> and thickness of container, which have decreased.

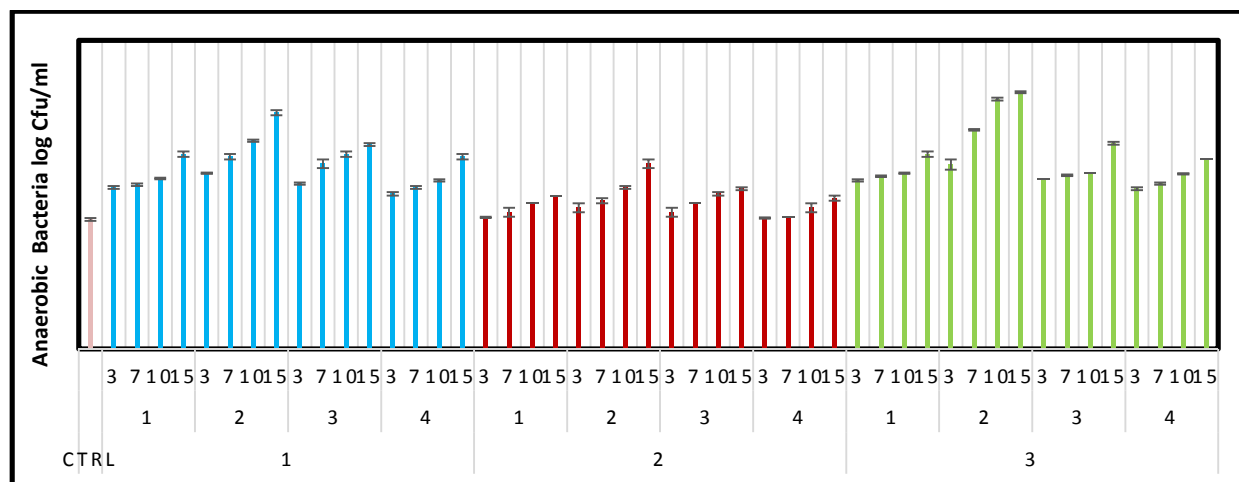


Fig 8-The effect of different concentration of gases, multi layers & storage times on anaerobic bacteria (Triple interaction) (log cfu/ml)

## DISCUSSION

The results of this study showed that CO<sub>2</sub> 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 were 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 anaerobic 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 Anaerobic bacteria in packed fresh ostrich meat belonged to layer: 2 (4-layers) under gas composition CO<sub>2</sub> 70% after control condition, but highest amount belonged to layer: 3(3-layers) under CO<sub>2</sub> 30%, and then vacuum. Because of the thickness and of type gas atmosphere, which were affected on growth of anaerobic bacteria till 15 days.

2- Anaerobic 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 effect of modified atmosphere storage on clostridium botulium growth and spoilage micro flora of salmon fillets, the results of anaerobic count were similar to these results.Grua, 1983 , indicated that due to microbial growth in different packed meat using multilayer films under vacuum condition in 0°C during 12 week , the results of anaerobic bacteria count were corresponded with these results .Ohlsson *et al.*,2002, due to research about Modified Atmosphere Packaging, (minimal processing technologies in food industry),results of this investigation were reliable. Vanderzant *et al.*, 2000 indicated that due to the shelf life and growth of anaerobic bacteria of packaged beef steak under vacuum and MAP condition in different barrier containers during 30 days were significant difference with ordinary condition as a control , the results of anaerobic bacteria count were similar to these results. Seydim *et al.*, 2006, due to the shelf life and growth of anaerobic bacteria of packed ostrich meat under vacuum and MAP condition, the results of anaerobic bacteria count were corresponded with these results.

Zand & Allahyari, 2013, concluded due to the influence of MAP and different multilayer flexible films on shelf life extension of candy bread during 3 week days , the results of anaerobic bacteria count under CO<sub>2</sub> %70 in 4-layer container were corresponded with these results. Zand & Sotoudeh ,2013 , indicated that due to, effect of MAP and multilayer flexible pouch for shelf life prolongation of chicken meal , the results of anaerobic bacteria count were corresponded with these results. 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,the best condition belonged to CO<sub>2</sub> %70, and results of anaerobic bacteria count were similar to these results. Zand, 2013, concluded due to shelf life extension of mashroom meal in multilayer flexible pouches 4-layer container was better than 3-layer during 60 days, results on anaerobic bacteria were corresponded with this



investigation. Zand, 2013, due to research about the shelf life prolongation of packed vegetables meal in multilayer flexible pouches 4-layer container was better than 3-layer during 60 days, results on anaerobic bacteria were similar to this study.

### CONCLUSION

The effect of MAP was not adequate but using this technique inactivated anaerobic microorganism growth without a significant adverse effect on food properties of ostrich meat samples. In the present study, it was concluded that, shelf life and growth of anaerobic bacteria of packed ostrich meat have been affected by different flexible multilayer 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. 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 two week.

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