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The usage of MAP for shelf life extension of packed spicy chicken meal

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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 for shelf life extension of spicy chicken meal at refrigerator (T= 4 ^{0}C). Ordinary condition as a control packaging were compared with two type of modified atmosphere packaging : {($N_2$70% + $CO_2$30%), ($N_2$30% + $CO_2$70%)} and vacuum conditions in this project .These samples (spicy chicken meal) were packaged into one kind of barrier flexible pouch" 3 layers with thickness 124 µ *(PET / AL / LLD)" under modified atmosphere packaging (MAP). Samples were performed microbial tests (Total count of bacteria, Lactobacilli count and Clostridium botulinum count), chemical test (pH) and sensory evaluation in different times. The usage of MAP was not adequate for controlling spoilage, but the spoilage process was delayed. The shelf life of chicken meals with spices under (30% Co_2 + 70% N_2) , (% 70 CO_2 +% 30 N_2) and also vacuum conditions in this container were 10 days. The best condition belonged to (N_2 30% + $CO_2$70%). Other hand sensory properties showed that increasing the amount of CO2 increased retention time, and can adversely affect the taste of spicy chicken meal under modified atmosphere. The PH variable has not decreased rapidly, in each treatments during storage times(28 days), while can be explained by characteristic of this multi layer flexible pouch with less water vapor and oxygen permeability, caused to control some chemical reactions in samples. Maximum change of pH was related to vacuum conditions, then treatment under % 30 CO_2 and the lowest changes belonged to treatment under % 70 CO_2 .*

Keywords: modified atmosphere packaging (MAP), shelf life, spicy chicken meal, flexible multi-layer films (3 layers)

INTRODUCTION

The modified atmosphere packaging (MAP) is a technique, which is widely used for shelf-life extension and improvement the quality of perishable foods stored at refrigeration temperatures [15].The ability of modified-atmosphere packaging for extending the shelf life of foods has been recognized for many years. Indeed, over 100 years ago [3,15]. Modified atmosphere packaging is the enclosure of a food, in a package in which the atmosphere has been changed by altering the proportions of carbon dioxide, oxygen, nitrogen, water vapour and trace gases. The process limits microorganism as well as biochemical activity. This modification is performed by gas flash packaging which oxygen is removed and replaced by a controlled mixture of gases [4]. MAP inhibits some microorganisms, so can increase the quality of variety foods. These products (spicy chicken meal) without a efficient processing are potential source of pathogens microorganism, specially mesophile and thermophile aerobic and anaerobic Clostridium and Bacillus, since the low acidity (pH 4-5) and high water activity of these packed meals creates an ideal environment for rapid microbial spoilage in this package [9, 10,17,18]. Although, thermal treatment (120 ^{o}C, 20 min) effectively destroys these microorganisms [10,17,18], has been used widely, proteins and some other

physiological substrates are inactivated, and consequently the flavor, taste, and contents of nutrients in foods are lost [17-21] .Other hands such treatment is carried out at high temperature at which shrinkages and leakages of pouches have been occurred that caused second contamination. For that reason, significant efforts are leading to the development of novel processing such as MAP [15, 17,18], which is proving to be able to inactivate spoilage microorganisms without significantly affect nutritional properties of several foods [1].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 concentration (%) of two gas $CO_{2/}$ N_2 in the head space as the independent variable for the gas atmosphere demonstrated that CO_2 exerts as an antimicrobial effect in the water-phase of the food product[3, 6, 15], therefore except the effect of intrinsic, extrinsic and processing parameters on the CO_2 solubility, the concentration of dissolved CO_2 in the water-phase of the food product should be incorporated in this study as independent variable [5] . Nitrogen (N2) is a non-reactive gas that has no smell or taste, unlike carbon dioxide, is not absorbed in food or water [3] . It is used as a filler gas to replace oxygen and thus prevent spoilage or to replace carbon dioxide and prevent package collapse [3] .The multi layers films have been used for packaging these meal is polymers or plastic films laminated with aluminum for packaging cooked meat and poultry instead of can [2,17-24] . Packaging materials need to be microwave transparent and have a high melting point; packages with some metal component can considerably change the food temperatures (critical process factor). The most common packages that have been tried are individual pouches made of microwave transparent rigid films such as polyethylene (LLD), and polyethylene terephtalate (PET), which are barrier films [11, 12,17-24], and metallic components present in a package, such as aluminum foil can dramatically influence on heating rates of the packaged food [13,19]. In this study, we investigate about the effects of modified atmosphere packaging; gas compositions with different concentrations of CO2/N2 and microbiological test; chemical test (PH); sensory evaluation [6], and the usage of one multilayer flexible pouch (3 layers) for shelf life prolongation of spicy chicken meal [13,14,17-19,24]. We want to prove MAP can substitute thermal processing in conservation industries, and have a lot of privilege [15] .

MATERIALS AND METHODS

2.1. Preparation of spicy chicken meal

Chickens (2 kg weight) were chosen for this experiment from local supermarket in Tehran -Iran. These Chickens were washed and cooked under Pressure (1 bar) for 30 min with sauce (1.5% Salt, 0.5% Pepper, 0.5% Turmeric, 0.5% Cinnamon) .Temperature was controlled in order to decrease to ambient temperature (T=25 ° C) .After cooling , cooked spicy chicken were cut into slices ,and samples were ready for packaging .Pouches contain 50 g , cooked spicy chicken [17,18,20,22] . 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 [17-24].

2.2.Modified atmosphere packaging

Henkelman packing machine, model Boxer-200A was used in this project. Samples were packed into one multilayer flexible pouch (3 layers) (PET/AL/LLD) under modified atmosphere (14,19). After packaging, samples were carried to refrigerator immediately, for determination shelf life, chemical test (PH) and microbiological tests (Total count of bacteria, Lactobacilli count and Clostridiums botulinum count) [24].

Fig 1. Modified atmosphere packaging(Model: Boxer-200A)

2.3. Microbial culture

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, RCA (Peptone from casein 10g/1000 ml; Meat Extract 10g/1000, Yeast Extract 3g/1000 ml, Starch 1g/1000 ml, glucose 5 g/1000 ml,l- cystein hydrochloride 0.5g/1000 ml, Sodium acetate 3g/1000 ml, Sodium chloride 5 g/1000 ml, Agar12.5g/1000 ml, Distillated water 1000 ml)Rein Clostridia is a culture Media for clostridium.CMM(Beef heart 454g/1000 , Proteose peptone 20 g/1000 ml,

glucose 5 g/1000 ml, Sodium chloride 5g/1000 ml, Sodium hydrochloride ½ 454 g/1000, Distillated water 1000 ml).Cooked Meat is an enrichment media for aerobic bacteria.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 water1000 ml). Peptone Yeast Extract Bromocresol Purple is an enrichment media for anaerobic bacteria [7, 8,17,18,20-24].MRS*(* Mann Rogosa and Sharpe agar) for Lactobacillus count [16].

Lactobacillus count in MRS culture

1 g of 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, while main sample was prepared by serial dilution(0.01,0.001…).Finally pour plate method was done in MRS culture (Mann Rogosa and Sharpe Agar), and incubated with 5% carbon dioxide for 4 days at 37 ° C in order to count Lactobacillus [7].

Total count of microorganisms in PCA culture

1 g of 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, while main sample was prepared by serial dilution(0.01,0.001…). Finally pour plate method was done in the PCA culture, in order to count total number of microorganisms which was incubated in 37 ° C for 3 days [7] .

***Clostridium botulinum* count in the RCA culture**

1 g of 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, while main sample was prepared by serial dilution(0.01,0.001…).Finally pour plate method was done in the RCA culture . Tubes (with gas pack) were put in the anaerobic jars, in order to count *Clostridium botulinum* which was incubated for 4 days at 37 ° C [7] .

2. 4-Samples packaging and storage

All pouches (unprocessed and processed spicy chicken meals), were put at refrigerator temperature (T= 4° C). Samples were packaged into one multilayer flexible films (3 layers) **.**Analytical characteristics of this barrier container were shown in table 1 [13,14,19].

Table 1- Analytical characteristics of container [13, 14, 19]

Sample	Layers	Thickness (µ)	Tensile of film (N)	Tensile of sealing film (N)	O.T.R ($ml/m^2.day$)	W.V.T.R ($g/m^2.day$)
PET\AL\LLD	12\12\100	124	93.22	58.8	0	0.11

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

2.5. Chemical tests

Measurements: PH

PH meter was adjusted with a buffered solution to 4 – 7.Sample (50 g) was uniformed ,and poured into 100 ml erlenmeyer flask .PH has been measured at ambient temperature (T=25 ° C) [24].

3. Statistical analysis

In order to describe the variables of this experiment, we must design a model to analysis relationship between type meal, type of cultures, and type of treatments .So comparison of data which was performed by the prism test [24].

RESULTS

4-1-Lactobacillus count

The Comparison of the growth lacto bacillus in MRS culture, during the storage time of samples at 4°C under vacuum and MAP conditions (30%CO_2 + 70% N_2), (70%CO_2 + 30% N_2) were shown in table 2. As you see , lacto bacillus grown in control samples after 7 days, however lacto bacillus were observed in the vacuum packaging after 21 days, and in different MAP condition was increased rapidly (non-countable) after 28 days [24] .

We have obtained these results, that the effect of gas components (30%CO_2 + 70% N_2), (70%CO_2 + 30% N_2) on growth of lacto bacillus was as the same. The growth of this bacteria can be occurred by second contamination (species). The variables were shown by different letter in each column had not significantly level with others. ($P<0.05$) [24].

Table 2. Lacto bacillus count in the MRS culture

28	21	14	10	7	3	2	1	treatment
10^9< c	10^9< c	10^9< c	10^9< c	10^9< c	10a	10a	10a	A
10^9< c	69×10^5 b	11a	11a	10a	10a	10a	10a	C1
10^9< c	11a	11a	11a	10a	10a	10a	10a	C2
10^9< c	11a	11a	11a	10a	10a	10a	10a	C3

A: control, C1: vacuum, C2:(30%co_2+70%N_2), C3: (70%co_2+ 30%N_2)

4-2-Total count of microorganisms

The growth of these bacteria's in control sample was observed after 3 days. Although growth of bacteria in C1, C2, C3 were observed after 14 days . As you see in table 3 ,the most growth of bacteria belong to vacuum condition then in 30%CO2 and 70% CO2. The variables were shown by different letter in each column had not significantly level with others.(P<0.05) [24] .

Table 3. Total count of microorganisms in PCA culture

28	21	14	10	7	3	2	1	Treatment
10^9< **f**	10^9< **f**	10^9< **f**	10^9< **f**	$4.58e_{+}$**007k**	**23×10^5**e	10a	10a	A
10^9< **f**	10^9< **f**	$4.43e_{+}$007i	11a	11a	11a	10a	10a	C1
10^9< **f**	$1.45e_{+}$008h	1.47e+007	11a	11a	11a	10a	10a	C2
10^9< **f**	22×10^5 c	25×10^4b	11a	11a	11a	10a	10a	C3

A: control, C1: vacuum, C2:(30%co2+70%N2), C3: (70%co2+ 30%N2)

4-3-*Clostridium botulinum* count

The growth of *Clostridium botulinum* in control sample was observed after 3 day, in vacuum condition and gas composition (% 30 CO2 +% 70 N2) was observed after 14 days, and there was significant difference between treatments C1 and C2 .The reason can be described by relation between the type of modified atmosphere in packages. The number of *Clostridium botulinum* was observed in vacuum condition was more than other MAP conditions. The growth of *Clostridium botulinum* in a gas mixture (70% CO_2 + 30% N_2) was observed after 21 days, as you see in table 4. The variables were shown by different letter in each column had not significantly level with others.(P<0.05) [24] .

Table 4. *Clostridium botulinum* count in the RCA culture

28	21	14	10	7	3	2	1	Treatment
10^9< **j**	10^9< **j**	10^9< **j**	2.75e+008h	3.96e+007f	27×10^5c	10a	10a	A
10^9< **j**	10^9< **j**	34×10^5d	10a	10a	10a	10a	10a	C1
10^9< **j**	1.38e+008g	11×10^5b	10a	10a	10a	10a	10a	C2
10^9< **j**	55×10^5e	10a	10a	10a	10a	10a	10a	C3

A: control, C1: vacuum, C2:(30%co_2+70%N_2), C3: (70%co_2+ 30%N_2)

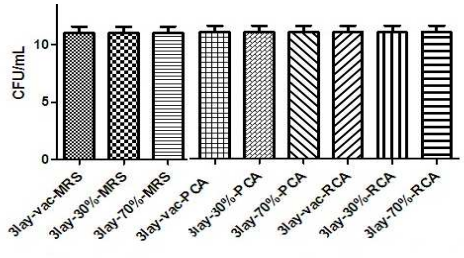

Fig 2- The growth of microorganisms (main samples) in different cultures after 1 day, under various concentration of co_2

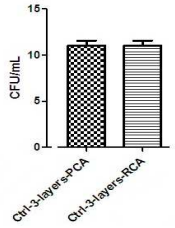

Fig 3- The growth of microorganisms (control samples) in different cultures after 3 days

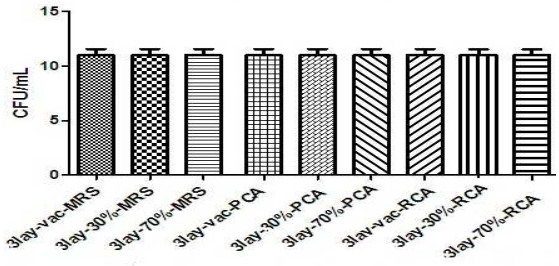

Fig 4- The growth of microorganisms (main samples) in different cultures after 3 days, under various concentration of co_2

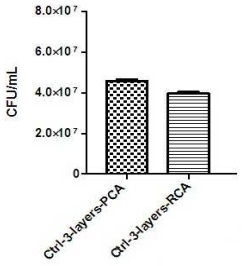

Fig 5- The growth of microorganisms (control samples) samples in different cultures after 7 days

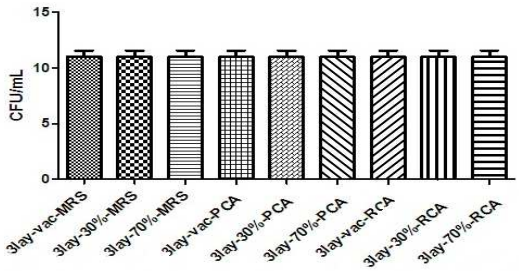

Fig 6- The growth of microorganisms (main samples) in different cultures after 7 days, under various concentration of co_2

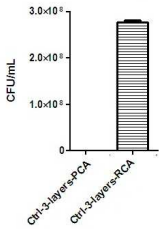

Fig 7- The growth of microorganisms (control samples) in different cultures after 10 days

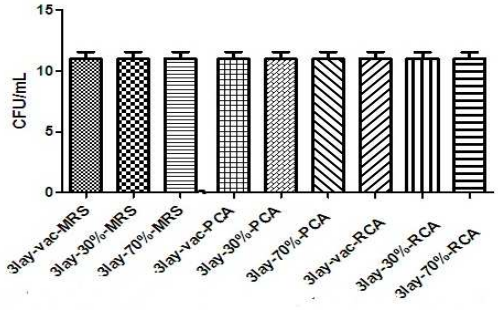

Fig 8- The growth of microorganisms (main samples) in different cultures after 10 days, under various concentration of co_2

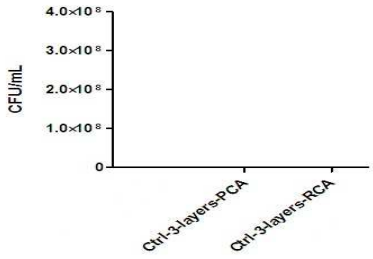

Fig 9- The growth of microorganisms (control samples) in different cultures after 14 days

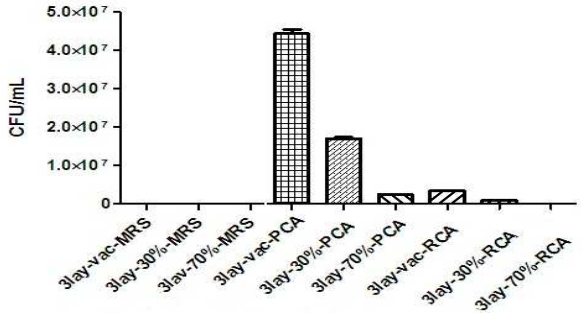

Fig 10- The growth of microorganisms (main samples) in different cultures after 14 days, under various concentration of co_2

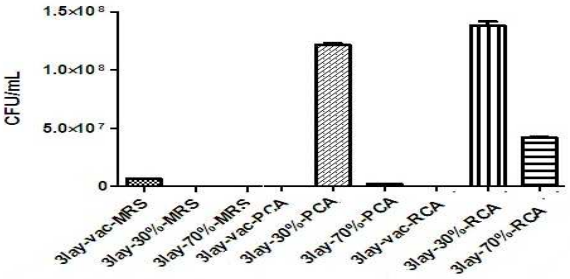

Fig 11- The growth of microorganisms (main samples) in different cultures after 21 days, under various concentration of co_2

The PH variable during storage times

The PH variable has not decreased rapidly , in each treatments during storage times. The experiments had been done in 28 days ,which there were not observed PH decreasing, while can be explained by characteristic of multi layer flexible pouches with less water vapor and oxygen permeability, caused to control some chemical reactions in samples, as you see in table 4.

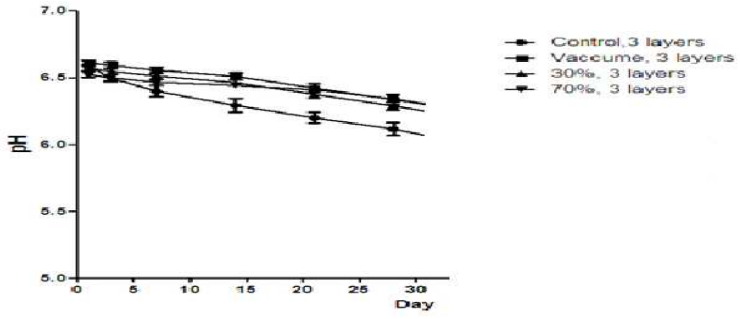

Fig 12. The PH variable of each treatments during storage times (28 days)

The passage of time lead to increase the number of microorganisms and accumulation of acid due to activity in the samples. Shelf life of samples had significantly level with pH ($p<0.05$). The PH variable had a significant level and effect on gas composition ($p<0.05$). The amount of CO_2 gas dissolved in water sample, cause to decrease the PH variable of each treatment. Maximum change of pH was related to treatment A, then treatment C1 and treatment C2 which have had the same trend and the lowest changes belonged to treatment C3[24].

Table 5. The PH variable of each treatments during storage times (28 days)

Shelf life						treatment
28	21	14	10	7	3	
6.11b	6.2b	6.29b	6.4b	6.49b	6.58a	**A**
6.33bB	6.42bA	6.51b	6.55bA	6.59b	6.61a	**B**
6.29bB	6.37bA	6.46b	6.51b	6.54b	6.57a	**C**
6.34bB	6.41b	6.44b	6.46b	6.5b	6.52a	**D**

A: control, C1: vacuum, C2:(30%co_2+70%N_2), C3: (70%co_2+ 30%N_2)

CONCLUSION

The shelf life of spicy chicken meal has evaluated according to the National Standard of Iran (ISIRIB2326). This parameter for low-acid foods (PH more than 4.6) which were packaged under vacuum , (30% Co_2 + 70% N_2) , and also (% 70 CO_2 +% 30 N_2) conditions into one kind of polymeric flexible pouch " 3 layers (124 µ)" were 10 days. The best condition belonged to (N_2 30% + $CO_2$70%) ,other hand sensory properties showed that increasing CO_2 increased retention time, and can adversely affect the taste of spicy chicken meal under modified atmosphere . The modified atmosphere packaging(MAP) weren't lead to stop spoilage completely .The effect of MAP was not adequate ,using this technique inactivated microorganism without a significant adverse effect on food properties and taste .The best shelf life of spicy chicken meal in ordinary conditions at refrigerator is 2 to 3 days. According to these results shelf life can be extended to 10 days[24].

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