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The influence of MAP and different multilayer flexible films on shelf life extension of candy bread

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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 two kinds of flexible multi-layer films has been studied for shelf life extension of candy bread at ambient condition (25^{0}C and 38 relative humidity). Ordinary condition as a control packaging were compared with three type of modified atmosphere packaging : {($N_2$70% + $CO_2$30%), ($N_2$30% + $CO_2$70%),($N_2$50% + $CO_2$50%)} and vacuum conditions in this project. Candy breads were packaged into two kind of barrier flexible pouches" 3 layers with thickness 124 µ (PET / AL / LLD) and ,4 layers with thickness 131 µ (PET / AL / PET/LLD)". Samples were performed microbial tests (Total count of bacteria's, Molds count), and sensory evaluation in different times (During 20 days).The usage of MAP was not adequate for controlling spoilage, but the spoilage process was delayed. The best condition belonged to condition under gas composition (Co_2 30%, N_2 70%) and packaging with 4 layers films in ambient temperature, which the shelf life of candy bread, was extended more 20 days. Sensory evaluation results showed that the samples which were packed in 3 layer films under normal atmospheric conditions (control) had the most rigid texture. However this reason (texture) could be attributed to protect the moisture of environment by multi-layer films for all treatments, in comparison with normal conditions. Other hand decreasing CO_2 (CO_2:30%) increased retention time, and can adversely affect the taste of this bread. The Analytical characteristics of these barrier containers (3,4 layers) were shown that efficiency of containers in 4 layers was better than 3 layers because the water vapor permeability of 4 layers was lower than 3 layers, and the usage of it is better for preserving candy bread during different period of experiment (after 4,8,12,16,20 days), under different gas compositions , and also for growth of microorganisms in different microbial cultures (Nutrient Agar, Sabouraud Dextrose Agar) **.**

Keywords: modified atmosphere packaging (MAP), shelf life, candy bread, flexible multi-layer films (3 ,4 layers)

INTRODUCTION

During the past 150 years, a high technological bakery industry has been developed. The small traditional bakeries have been replaced by a highly technological bakery industry .Bakery products have a very short shelf life and their quality depends not only on the process variables, but also on the time between baking and consumption [2]. The quality and shelf life of bakery products is usually reduced by chemical and physical changes which called staling. Staling process causes to reduce the acceptability of bakery products by consumers and involves some changes which have been occurred before spoilage of microorganisms .This is obvious , the control of staling process and second contamination of breads have been evaluated both by consumers and researchers [9] .The modified

atmosphere packaging (MAP) is a useful technique for various researches. However, it is well known that there is a non-thermal method for inactivation microorganism ,which is widely used for shelf-life prolongation, and improvement the quality of perishable foodstuffs such as meat, fishery, vegetables, and packed bakery products [1,3,17],and also there is no degradation of flavor and taste with heat denaturation of objectives [3,10, 26,27]. The ability of modified-atmosphere packaging for extending the shelf life of foods has been recognized for many years. Indeed, over 100 years ago [1,17].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 vapor 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 controlled gas compositions [3]. MAP inhibits some microorganisms, so can increase the quality of variety foods.Candy bread is one of the most common flat bread in Iran, which has a relatively short shelf life. For this reason, significant efforts are leading to the development of novel processing such as MAP [17,18,26-27], that is proving to be able to inactivate spoilage of microorganisms without significantly affect nutritional properties of several foods. Although CO2 is not known to be lethal to microorganisms, it has shown both bacteriostatic and fungistatic properties and will hinder the growth of certain aerobic organisms [16].So there is an increasing demand for storage of bread in modified atmospheres, which is most often composed of CO2 alone or mixtures of CO2 and N2 [15].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 [1, 17,18,26,27], 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 [4]. Nitrogen (N2) is a non-reactive gas that has no smell or taste, unlike carbon dioxide, is not absorbed in food or water [1]. It is used as a filler gas to replace oxygen and thus prevent spoilage or to replace carbon dioxide and prevent package collapse [1] .The multi layers films have been used for packaging this bread is polymers or plastic films laminated with aluminum [6-8,19-21] . 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 [6-8,11, 12, ,19-20],and metallic components present in a package, such as aluminum foil , can dramatically influence on heating rates of the packaged food [6-8,11, 12, 19-20]. In this study, we investigate about the effects of modified atmosphere packaging; gas compositions with different concentrations of CO2/N2 and microbiological test [26], and the usage of two multilayer flexible pouches (3,4 layers) [13,14,21-27] for shelf life prolongation of a one candy bread [21-27]. We want to prove MAP can extend the shelf life of bakery products [26,27], and these flexible pouches can also improve the marketability of breads, for easy usage of the package, and great importance for best sell of such items especially for military foods [21].

MATERIALS AND METHODS

Table 1-Different condition of packaging of candy breads

No	Containers	Treatments
Treatment 1	PET/AL/LLD	CO_2:70% N_2: 30%
Treatment 2	PET/AL/LLD	CO_2:30% N_2: 70%
Treatment 3	PET/AL/LLD	CO_2:50% N_2: 50%
Treatment 4	PET/AL/LLD	Vacuum
Treatment 5	PET/AL/LLD	Control
Treatment 6	PET/AL/PET/LLD	CO_2:70% N_2: 30%
Treatment 7	PET/AL/PET/LLD	CO_2:30% N_2: 70%
Treatment 8	PET/AL/PET/LLD	CO_2:50% N_2: 50%
Treatment 9	PET/AL/PET/LLD	Vacuum
Treatment10	PET/AL/PET/LLD	Control

2.1. Preparation of candy bread

These candy breads were chosen for this experiment, prepared in a traditional bakery in Tehran -Iran. The raw materials for the baking these breads included: confectionery flour (Corn Flour company, Iran), yeast (Iran Molasses Company, Iran) and salt (Gift Company, Iran). The temperature of breads must be controlled in order to decrease

to ambient temperature (T=25 ° C) .After cooling , samples of bread were ready for packaging so, were divided into small pieces and placed under sterile conditions inside the containers.Pouches contain 50 g , candy bread [22-27]. This research was conducted to 10 treatments in 3 run with different containers(3 layers and 4 layers) and different condition of modified atmosphere packaging (5 kinds), as you see in table 1[22-27].

2.2. Modified atmosphere packaging

Henkelman packing machine, model Boxer-200A was used in this study. Samples were packed into two multilayer flexible pouch (3,4 layers) [13,14,21] under modified atmosphere according to table 1. After packaging, samples were put in ambient condition (room temperature), for determination shelf life, and microbiological tests (Total count of bacteria, and Mold counts)[26,27].

A **B** **C**

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

2.3. Microbial culture

NA(Peptone from meat 5g/1000 ml, Meat Extract 3 g/1000 ml, Agar 12g/1000 ml, Distillated water 1000 ml),Nutrient agar is a general media for aerobic bacteria; SDA(Peptomycol 10g/1000 ml, Glucose 40g/1000 ml, Agar 12g/1000 ml) Sabouraud Dextrose Agar is a general media for mold [5,26,27].

Total count of microorganisms in NA culture and Mold count in the SA culture

Total count of microorganisms were performed by pour plate counts on NA (Nutrient Agar) culture. For this reason, temperature of liquid NA culture was reached to 45 ° C , and 1 ml of different dilutions mixed with 15 mL of NA in Petri dish. After shutting down agar, samples were placed at room temperature for 48 to 72 hours. Total count of mold were done by pour plates in triple run on SDA (Sabouraud Dextrose Agar) culture. The colonies were counted after incubating 3 days in ambient temperature. Plates and pipette were sterilized before microbial testing at 160 ° C for 2 h. Medium was sterilized in autoclave (at 121 ° C for 20 min). Preparation of serial dilutions of samples was done according to CFU method. 1 g of sample was weighed under the microbial laboratory hood, and was crushed in 10 ml of ringer's solution. Samples, 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 (0.01,0.001,0.0001,…). Microbial testing were done according to the ISIRI regulation (ISIRI regulation No 997) Cultures (NA,SDA) were taken from Merck Company (Germany) [5,26,27].

2. 4-Samples packaging and storage

All pouches (unprocessed and processed candy breads), were put at room temperature (T= 25° C). Samples were packaged into two multilayer flexible films**.** Analytical characteristics of these barrier containers were shown in table 2[13,14,21].

Table 2- Analytical characteristics of containers [13, 14, 21]

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\PET\LLD	12\7\12\100	131	104.61	61.03	0	0.089
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

3. STATISTICAL ANALYSIS

In order to describe the variables of this experiment, we must design a model to analysis relationship between candy bread , type containers(2 kind), type of cultures(2 kind), and type of treatments(5 kind), during different storage times . So comparison of data which was performed by Duncan's new multiple range test , with confidence level of 95% (P <0.05) .Software Ver 1.41 (MstatC Switzerland) was used to perform this test [26,27].

RESULTS

According to result of table 3 (Analysis of variance mean squares in response to treatments) .The effect of different microbial cultures, different gas compositions, different plates, different multilayer flexible films, and the double interaction between: environment and times, environment and gas compositions, environment and plates , gas compositions and times , plates and times, containers and times, gas compositions and plates, gas compositions and containers, and also triple interaction between: (A) times , gas compositions, environment ;(B) environment, plates, times;(C) environment, plates, gas compositions;(D)times, gas compositions ,plates (E)times, gas compositions, containers (F)times, plates, containers(G) plates, gas compositions , containers ,on number of colonies had significant effects (P <0.01).

Table 3- Analysis of variance mean squares (number of bacteria) in response to treatments

No of microorganism	Freedom	Treatment
1761718**	1	culture
2398612**	4	time
4968997**	4	gas
473312.1**	4	plate
73836.38**	1	Container(3,4 layer)
46132.56**	4	time*culture
575295.9**	4	gas*culture
30304.91**	4	plate*culture
915.72 ns	1	container*culture
298406**	16	gas*time
39181.29**	16	plate*time
534639.2**	16	plate*gas
26631.1**	4	container*time
20691.42**	4	container*gas
2755.11 ns	4	container*plate
131240.2**	16	gas* time* culture
12905.38**	16	plate* time* culture
22012.79**	16	plate*gas* culture
1035.12 ns	4	container*gas* culture
530.27 ns	4	container*plate* culture
914.01 ns	4	container* time* culture
63823.72**	64	plate*gas* time
23657.38**	16	container*gas* time
5682.75**	16	container*plate* time
6797.42**	16	container*plate*gas
1578.78	1240	Error
28.93	-	Variance Index

*, ** *had significant effect at level 1 and 5% ,and ns had no significant effect*

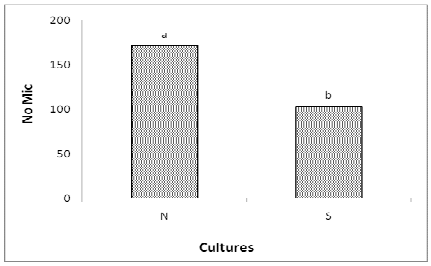

Fig 2.The effect of different cultures on growth of bacteria's.
(N =, S =) means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α).

As you see in figure 2, the effect of different cultures on growth of colonies were shown. In this study in order to count molds and total bacteria's, SDA culture, and NA culture were used . In fig above, was observed the relationship between two cultures and number of colonies which had a significant difference (P <0.01) ,and the number of colonies in SA culture was less than PCA culture .

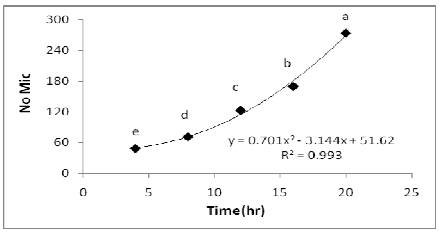

Fig 3.The effect of different storage times on growth of bacteria's.
(h) means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α).

As you see in figure 3, the effect of different storage times (4-20 days) on growth of colonies in room temperature were shown. The number of microorganism were reported by no : 0-500.The lowest number of colonies was observed after 4 days(e),however the highest number belonged to day 20(a).During the last days of storage times (b-a), the growth of colonies were doubled.

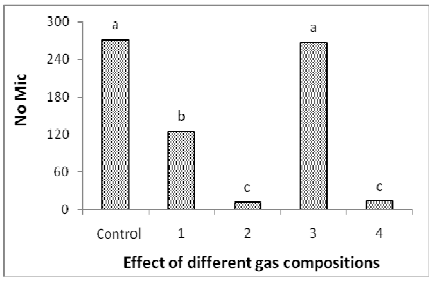

Fig 4.The effect of different gas compositions on growth of bacteria's, means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α).

As you see in figure 4, the effect of different gas compositions on growth of colonies were shown by no: 0-4(control,Co_2 70%; Co_2 30% ; Co_2 50% Co_2 0%). The lowest number of colonies was observed in treatments 2 and 4 ,however the highest number of colonies belonged to treatments 0 and 3

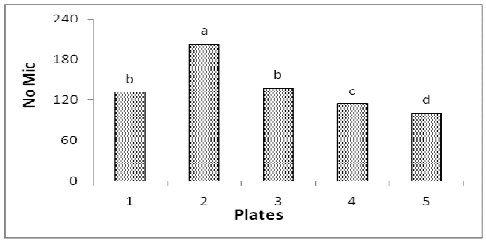

Fig 5.The effect of different plates on growth of bacteria's,

means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α).

As you see in figure 5, the effect of different plates on growth of colonies were shown as a direct effect by plates no: 1-5 . The lowest number of colonies was observed in plates 5 and 4 ,however the highest number of colonies belonged to plate 2 ,and you could found significant effect in all of plates.

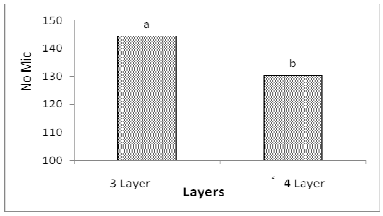

Fig 6.The effect of different containers on growth of bacteria's,

means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α).

As you see in figure 6, the effect of different containers on growth of colonies were shown by 3 and 4 layers containers. The lowest number of colonies was observed in 4 layers container ,however the highest number of colonies belonged to 3 layers container.

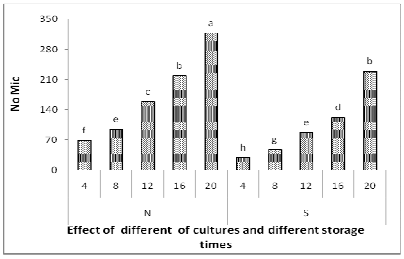

Fig 7.The effect of different cultures and different storage times on growth of bacteria's

(N =, S =), means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α).

As you see in figure 7, the effect of different cultures and different storage times on growth of colonies were shown by cultures(NA;SA) and different storage times (4-20 days). The lowest number of colonies was observed after 4 days in NA and SA cultures too ,however the highest number of colonies belonged to last day (after 20 days) in NA culture and also SA culture.

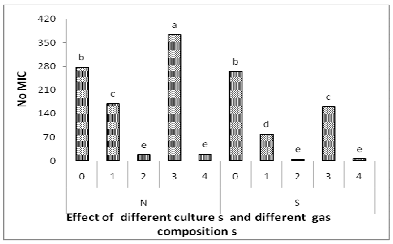

Fig 8.The effect of different cultures and different gas compositions on growth of bacteria's

(N =, S =), means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α).

As you see in figure 8, the effect of different cultures and different gas compositions on growth of colonies were shown by no 0-4(control,Co_2 70%; Co_2 30% ; Co_2 50%; Co_2 0%) for gas compositions and cultures (NA;SA). The lowest number of colonies was observed in treatments 2 and 4 in NA culture and SA culture too , however the highest number of colonies belonged to treatment 3 in NA culture and treatment 0 in SA culture.

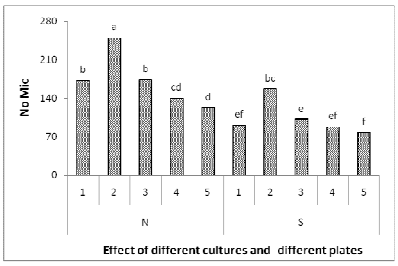

Fig 9.The effect of different cultures and different plates on growth of bacteria
(N =, S =), means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α).

As you see in figure 9, the effect of of different cultures and different plates on growth of colonies were shown by cultures (NA;SA) and plates (1-5). The lowest number of colonies was observed in plate 5 in NA and SA cultures ,however the highest number of colonies belonged to plate 2 in NA culture and SA culture too.

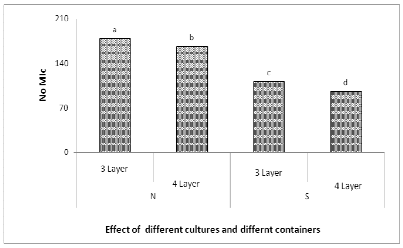

Fig 10.The effect of different cultures and different containers on growth of bacteria's,
means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α).

As you see in figure 10, the effect of different cultures and different containers on growth of colonies were shown by cultures(NA;SA) and containers (3,4 layers). The lowest number of colonies was observed in 4 layers container in NA and SA culture ,however the highest number of colonies belonged to 3 layers container in NA culture and also SA culture.

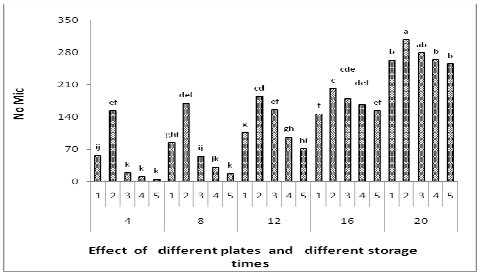

Fig 11.The effect of different plates in different storage times on growth of bacteria's,
means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α).

As you see in figure 11, the effect of of different plates and different storage times on growth of colonies were shown by plates (1-5) and storage times (4-20 days). The lowest number of colonies was observed in plate 5 in each storage times(after 4,8,12,16,20 days) ,however the highest number of colonies belonged to in plate 2 during each period of storage times(after 4,8,12,16,20 days).

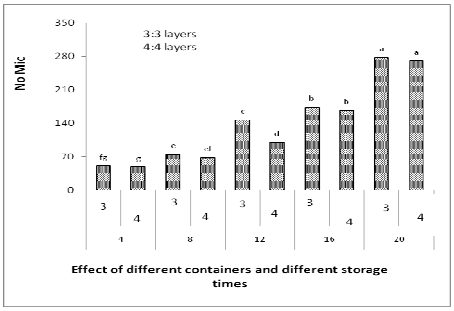

Fig 12.The effect of different containers in different times on growth of bacteria's(control),
means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α).

As you see in figure 12, the effect of different containers and different storage times on growth of colonies were shown by containers (3,4 layers) storage times (4-20 days). The lowest number of colonies was observed in 4 layers container, however the highest number of colonies belonged to 3 layers container during each period of storage times (after 4,8,12,16,20 days).

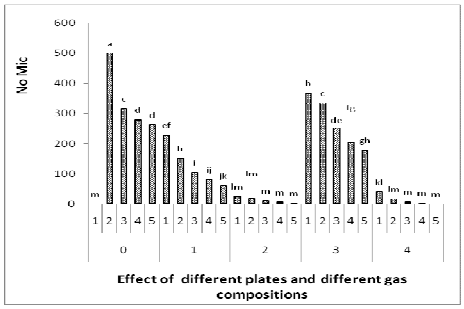

Fig 13.The effect of different plates and different gas compositions on growth of bacteria,
means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α).

As you see in figure 13, the effect of different plates and different gas compositions on growth of colonies were shown as by plates (1-5), no 0-4(control,Co_2 70%; Co_2 30% ; Co_2 50% Co_2 0%) for gas compositions . The lowest number of colonies was observed in plate 5, however the highest number of colonies belonged to plate 1 in each gas compositions .

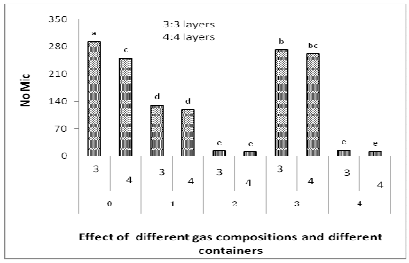

Fig 14.The effect of different containers and different gas compositions on growth of bacteria's,
means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α).

As you see in figure 14, the effect of of different gas compositions and different containers on growth of colonies were shown by no 0-4(control,Co_2 70%; Co_2 30% ; Co_2 50% Co_2 0%) for gas compositions and containers (3,4 layers). The lowest number of colonies was observed in 4 layer container , however the highest number of colonies belonged to 3 layer container in each gas compositions .

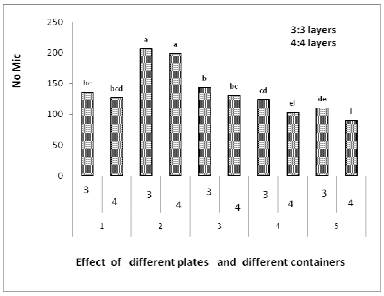

Fig 15.The effect of different containers and plates on growth of bacteria's,
means that at least one common letter had not significantly difference (least significant difference test (LSD), 5% = α)

As you see in figure 15, the effect of of different plates and different containers on growth of colonies were shown by plates (1-5) and containers (3,4 layers). The lowest number of colonies was observed in 4 layer container ,however the highest number of colonies belonged to 3 layer container in each plates ,too.

CONCLUSION

The shelf life of candy bread has evaluated according to the National Standard of Iran (ISIRIB 997) . Samples were packaged under vacuum,, and {($N_2$70% + $CO_2$30%), ($N_2$30% + $CO_2$70%),($N_2$50% + $CO_2$50%)} conditions into two kinds of barrier flexible pouch "3 (124 µ)and 4 layers (131 µ)" . Candy breads were performed microbial tests (Total count of bacteria, Molds count), and sensory evaluation during 20 days .The usage of MAP was not adequate for controlling spoilage, but the spoilage process was delayed . The best condition belonged to condition under gas composition (Co_2 30%, N_2 70%) and packaging with 4 layers films in ambient temperature, which the shelf life of candy bread, was extended more 20 days. Sensory evaluation results showed that the samples which were packed in 3 layer films under normal atmospheric conditions had the most rigid texture. However this reason (texture) could be attributed to protect the moisture of environment by multi-layer films for all treatments, in comparison with normal conditions. Other hand decreasing CO_2 (CO_2:30%) increased retention time, and can adversely affect the taste of this bread. The Analytical characteristics of these barrier containers (3,4 layers) were shown that efficiency of containers in 4 layers was better than 3 layers because the water vapor permeability of 4 layers was lower than 3 layers, and the usage of it is better for preserving candy bread during different period of experiment (after 4,8,12,16,20 days), under different gas compositions , and also for growth of microorganisms in different microbial cultures (Nutrient Agar, Sabouraud Dextrose Agar) **.**

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REFERENCES

[1] Church, N., **1994**,*Trends in food science & technology*, 5(11):345-352.
[2] Decock, P., & Cappelle, S. ,**2005**, *Trends in Food Science & Technology*, 16, 113–120.)
[3] Desrosier NW, Desrosier JN. ,**1977**, The technology of food preservation: AVI Publishing Company, Inc.
[4] Devlieghere F, Van Belle B, Debevere J., **1999** , *International journal of food microbiology*,46(1):57-70.
[5] Institute Of Standards And Industrial Research Of Iran (ISIRI), **2005** ,No 997, Bakery Products , 1 nd .Revision, Karaj – Iran
[6] Mailova E ; Zand N., **2010**,Combined packaging material flexible packs characteristics dependence on changes of components composition and quantity, Processing s of Engineering Academy of Armenia ,Vol. 7, No. 1, (Article in Russian) Republic of Armenia ,P: 129-132
[7] Mailova E , Zand N.,**2010**,The strength of the weld seams of flexible packages depending on the sealing mode ,*Agro science* , No. 1-2 , (Article in Russian) Republic of Armenia ,P:73-77
[8] Mailova E. , Zand N.,**2010**,The influence of thermal processing on hermit city of flexible packaging ,*Agronomy* and *Agro ecology* ; No. 2, (Article in Russian) Republic of Armenia,P:96-99

[9] Martin, M. L., Zeleznak, K. J. and Hoseney, R. C. ,**1991**, *Cereal Chemistry*, 68: 498-502.
[10] Mortazavi A, Kasshani N, Ziaolhagh H., **2002**,Food Microbiology (WC Frazier), Ferdowsi University Press
[11] O'Meara, J. P., Farkas, D. F. and Wadsworth, C. K., **1977**, Flexible pouch sterilization using a combined microwave-hot water hold simulator. Natick, MA. US Army, Natick Research & Development Lab, Unpublished Report. (PN) DRYNM P:77-120
[12] Ohlsson, T. ,**1991,** Development and evaluation of a microwave sterilization process for plastic pouches. 8th World Congress of Food Science and Technology. Toronto, CA. Sept. 29- Oct. 4
[13] Paine FA., Paine HY., **1992,** A handbook of food packaging: Springer
[14] Plastic mashine alvan(P.M.A) com,**2006**, Tehran ,Iran (www.printopack.com)
[15] Rasmussen, P. H., Hansen, A. ,**2000,** Lebensmittel-Wissenchaft & Technologie. 34: 487-491.
[16] Seiler, D. A. L. ,**1989**, USA: Food & Nutrition Press, Inc. pp 119-133.
[17] Young H., **1987**,Food Microbiology,4(5):317-327.
[18] Young H, Young A, Light N. ,**1987**,Food Microbiology,4(4):317-327.
[19] Zand N.; Mailova E., **2009**,The study of The barrier properties of the combined film, Annals of Agrarian Science Vol. 7,No. 3 , (Article in Russian) Republic of Georgia,P:94-95
[20] Zand N.,**2011**,Correlation of plastic multilayer packaging films mechanicals properties with composition and percentage of their composition, Information technologies and management,
No. 1, (Article in Russian) Republic of Armenia ,P:232-234
[21] Zand N. ,**2011**, *Annals of Biological Research*, vol. 2, issue 2 , P: 488-501
[22] Zand N. ,**2011**, *Annals of Biological Research*, vol. 2, issue 3 , P: 442-452
[23] Zand N. ,**2011**, *Annals of Biological Research*, vol. 2, issue 4, , P: 398-407
[24] Zand N. ,**2013**, *European Journal of Experimental Biology*, vol. 3, issue 2, P:598-607
[25] Zand N. ,**2013**, *European Journal of Experimental Biology*, vol. 3, issue 3, P:246-253
[26] Zand N. ,Sotoudeh B., **2013**, *European Journal of zoological Research* , vol. 2, issue 2, P:26-33
[27] Zand N,.Sotoudeh B., **2013**, *Annals of Biological Research*, vol. 4, issue 6, , P: 175-181