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Effect of Modified Atmosphere Packaging and Multi-layer Flexible Films on Sensory Evaluation of Smoked Kutum Fish (*Rutilus frisii kutum*)

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ABSTRACT

In this study the effect of different concentrations of three gas mixture (carbon dioxide, nitrogen,oxygen) ,and also vacuum conditions and flexible multi-layer films has been studied on sensory evaluation of smoked kutum fish (Rutilus frisii kutum) at ambient condition (T= 25 ^{0}C) . Ordinary condition as control packaging were compared with four types of modified atmosphere packaging: ($N_2$70%+ $CO_2$30%), ($N_2$30% + $CO_2$70%), (45%CO2+45%N2+10%O2), and vacuum conditions, in this project. Smoked kutum fish were packaged in 3 kinds of flexible multi-layer containers under modified atmosphere packaging,{3-layers($PET_{(12)}$/$AL_{(12)}$/$LLD_{(100)}$), 4-layers ($PET_{(12)}$/$AL_{(7)}$/$PET_{(12)}$/$LLD_{(100)}$),and 3- layer($PET_{(12)}$/$AL_{(7)}$/$LLD_{(100)}$ }. Samples were performed sensory evaluation, in different times, with 15 treatment ,3 run, statistical analysis and comparison of data, were done by software SAS (Ver:9/1) and Duncan's new multiple range test, with confidence level of 95% (P <0.05). The shelf life of smoked kutum fish according to organoleptic properties were reported under conditions 1,2,3 in 4-layers , 60,58,45 days and in vacuum conditions were about 40 days, in 3-layers (AL:12) , under conditions 1,2, 3 and vacuum conditions were 55,50,40,35 days, with 3-layers(AL:7),under conditions1,2,3 were 45,40,35 days and in vacuum conditions were 30 days. During the period of this experiment (60 days) sensory properties of samples in various conditions, had significant differences. Sensory evaluation showed that increasing CO2 concentration extended shelf life, and could affected texture,color, appearance ,smell, taste of smoked kutum fish. Especially taste & texture had better result. under gas composition ($N_2$30%+ $CO_2$70%) with 4- layers , since steam permeability of 4-layer was less than 3-layer According to these results could be concluded the best condition for sensory evaluation belonged to treatment under modified atmosphere CO2 70% with flexible pouch {$PET_{(12)}$/$AL_{(7)}$/$PET_{(12)}$/$LLD_{(100)}$}.

Keywords: modified atmosphere packaging (MAP), sensory evaluation, flexible multi-layer films, smoked kutum fish (*Rutilus Frisii Kutum)*

INTRODUCTION

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 , improvement the quality of perishable foodstuffs such as meat, fishery, vegetables, and packed bakery products [2,6,19,29-32], and also there is no degradation of flavor and taste with heat denaturation of objectives [6,18,19,29-32]. The ability of modified-atmosphere packaging for extending the shelf life of foods has been recognized for many years [1-3,19, 29-32]. Modified atmosphere packaging is an enclosure of 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 [6-8,19, 29-32]. This modification is performed by gas flash packaging which oxygen is removed and replaced by controlled gas compositions [18]. MAP inhibits some microorganisms, so can increase the quality of variety foods such as sea foods [5,15,19,29-32]. Kutum

fish (*Rutilus frisii kutum)*, is a famous fish which is distributed from Turkmenistan to Azerbaijan and Iran along the Caspian Sea. It is one of the economically important fish in the region [9]. Kutum fish is constituted about 78% of bony fish harvest and about 76.6% of the whole income of fishermen in the 2008-2009 during fishing season in the southern part of Caspian Sea [9]. Smoked fish, is a kind of processed fish that is prepared by cold smoke (25-30 ^{0} C) or hot smoke [80 ^{0} C) [4,12].Smoked kutum fish (cold type) has short shelf life. For this reason, significant efforts are leading to the development of novel processing such as MAP [4,13,14,19,29-32], 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 micro-organisms [13-15]. So there is an increasing demand for storage of this fish in modified atmospheres, which is most often composed of CO2 alone or mixtures of CO2 and N2 [2,5 ,19,29-32]. 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 three gas CO2/ N2/ O2 in the head space as the independent variable for the gas atmosphere demonstrated that CO2 exerts as an antimicrobial effect in the water-phase of the food product [3,7,19,29-32], 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 [7]. 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, and also oxygen (O2) prevents growth of anaerobic bacteria [2,8,19,29-32]. The multi-layer films have been used for packaging this fish is plastic films laminated with aluminum [22,23,27,28,19,29-32]. 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) [24-26].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 [16,17,19,29-32], and metallic components present in a package, such as aluminum foil , can dramatically influence on heating rates of the packaged food [18, 19, 26,27, 29-32]. The Analytical characteristics of these barrier container (3 & 4 layers) caused to control some chemical reactions in samples and preserved sensory properties , prolonged shelf life by less water vapor and oxygen permeability [22,23,27,28,19,29-32]. In this study, we investigate about the effects of modified atmosphere packaging with different concentrations of CO2/N2/O2 , and the usage of three multilayer flexible pouches (3 & 4 layers) on sensory properties of smoked kutum fish (*Rutilus Frisii Kutum)* during 60 days [30,32]. We want to promote MAP and these multilayer flexible films can extend the shelf life of smoked fish products [22,23,27,28,19,29-32] , and also improve the sensory properties of samples [19,24-26, 29-32].

MATERIALS AND METHODS

Preparation of samples (smoked kutum fish)

5, smoked kutum fish (prepared by cold smoke recently) each weighing 1.5 kg from a distribution center of fish in Tehran were bought for this study. The head and tail of samples, were isolated and then samples of fish, were divided into small pieces (60 g) and placed under sterile conditions inside there multilayer containers. This research was conducted to 5 treatments in 3 run{($N_2$70% + $CO_2$30%),($N_2$45% + $CO_2$45%+ $O_2$5%), ($N_2$30% + $CO_2$70%), vacuum condition , ordinary condition}. Temperature was controlled in order to decrease to ambient temperature (T=25 °C). 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 [19,24-26,29-32].

Modified Atmosphere Packaging

Henkelman packing machine, model Boxer-200A was used in this project. Samples were packed into three multilayer flexible pouches 3-layer ($PET_{(12)}/AL_{(12)}/LLD_{(100)}$) ,and 4-layer ($PET_{(12)}/AL_{(7)}/PET_{(12)}/LLD_{(100)}$), and 3-layer ($PET_{(12)}/AL_{(7)}/LLD_{(100)}$) under modified atmosphere . After packaging, samples of smoked kutum fish were put in ambient condition (room temperature), for evaluation sensory properties during 60 days [19, 29-32].

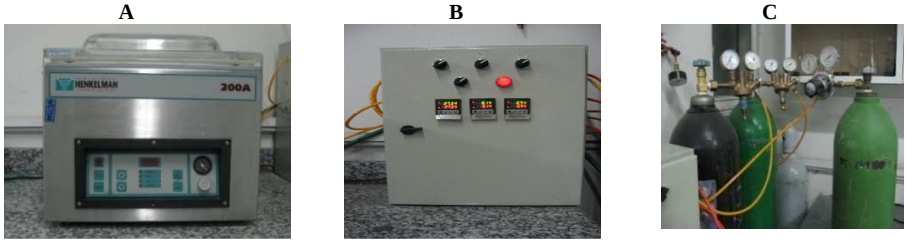

Fig 1. (A) Modified atmosphere packaging, (B) gas analyzer, (C) gas flash tank(Model: Boxer-200A) (19, 29-32).

Samples packaging and storage

All pouches (smoked kutum fish), were put in at ambient condition (T= 25 ^{0}C).Analytical characteristics of these barrier containers were shown in table 1 [19,24-26,29-32].

Table 1- Analytical characteristics of containers (19,24-26,29-32).

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

Sensory properties

Evaluation of sensory properties (appearance, color, texture, Smell, taste) has been designed according to numerical parameter for each samples, as you see in table 2. These properties have been described with marking system. Best Marking System was 1-5 (1-Very Good; 2-Good; 3-Medium; 4- Weak; 5- Very Weak). At least 100 members of panel (tester) were chosen for this investigation. The usage of multiple comparison test, were studied simultaneously, and specific test (marking system) for each samples, had done after 15,30,45,60 days of packaging. In multiple comparison test, smoked kutum fish with the code number were given to panel, and they were asked to compare the control smoked kutum fish (Ordinary condition samples) and coded smoked fish (under modified atmosphere and vacuum), and filled application form (table below) [24-26,30,32].

Table 2 .Evaluation of sensory properties (appearance, color, texture, taste, smell) of samples [30,32]

Notes	Sensory properties					Product
	color	Smell	Taste	Texture	Appearance	

Very Weak	Weak	Medium	Good	Very Good
5	4	3	2	1

STATISTICAL ANALYSIS

In order to describe the variables of this experiment, we must design a model to analysis relationship between smoked kutum fish, and type of treatments on sensory properties during different storage times. Statistical analysis of data, was performed by 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) [24-26,29-32].

RESULTS

Sensory properties of smoked kutum fish in different conditions

According to table 3, the effect of storage times (days) and the effect of different gas compositions and also the effect of different containers on sensory properties had significant level (P <0.01), although the effect of layers on smells and colors had a significant difference level (P <0.05).

Table 3-Analysis of variance mean squares traits in response to treatments

Smell	Taste	Texture	Color	Appearance	Degrees of freedom	Variables
0.43*	1.91**	2.46**	0.60*	1.86**	2	**layer**
25.54**	29.12**	28.54**	26.06**	27040**	4	**gas**
0.051^{ns}	0.07^{ns}	0.08^{ns}	0.13^{ns}	0.08^{ns}	8	**Gas*layer**
151.01**	169.62**	167.99**	171.24**	172.75**	3	**Time(day)**
0.34*	0.09^{ns}	0.07^{ns}	0.11^{ns}	0.16^{ns}	6	**Layer*time(day)**
2.42**	2.67**	2.94**	2.62**	2.88*	12	**Gas*time(day)**
0.07^{ns}	0.21*	0.10^{ns}	0.10^{ns}	0.06^{ns}	24	**Layer*gas*time(day)**
0.11	0.12	0.14	0.14	0.12	239	**errors**
9.23	10.03	10.89	10.56	9.97	-	**Variance Index (CV)**

According to tables, 4 & 5,the best mark of sensory properties (appearance, color , texture , smell, taste) belonged to 4-layers , under (70% CO_2 + 30% N_2) conditions , and the worst mark observed in 3- layers(AL:7) with ordinary condition due to the thickness and type of gas composition.

Table 4: Comparison of sensory properties (appearance, color, texture, smell, taste) on different layers

Smell	Taste	Texture	Color	Appearance	Treatments / layers
3.701[b]	3.510[b]	3.403[b]	3.561[b]	3.430[b]	**layer1: 3-layers(AL:12)**
3.851[a]	3.644[a]	3.661[a]	3.692[a]	3.661[a]	**layer 2: 3- layers(AL:7)**
3.693[b]	3.373[c]	3.383[b]	3.560[b]	3.424[b]	**layer 3: 4- layers**

Table 5: Comparison of sensory properties (appearance, color, texture, smell, taste) on different gas compositions

Smell	Taste	Texture	Color	Appearance	Treatments / gas
2.652[e]	2.40[e]	3.381[e]	2.520[e]	2.423[d]	**CO_2 70% +N_2 30%**
3.872[d]	3.601[d]	3.550[d]	3.702[d]	3.650[c]	**CO_2 30% + N_2 70%**
3.751[c]	3.35[c]	3.343[c]	3.574[c]	3.351[b]	**CO_2 45% + N_2 45%,+ 10% O_2**
4.171[b]	4.05[b]	3.981[b]	4.021[b]	4.001[a]	**vacuum**
4.303[a]	4.131[a]	4.130[a]	4.220[a]	4.100[a]	**control**

According to table 6, the effect of different storage times on smoked kutum fish were shown. The lowest mark of sensory properties (appearance, color, texture, smell, taste) of samples was observed after 15 days and highest mark after 60 days.

Table 6: Comparison of sensory properties (appearance, color, texture, Smell, taste) on different days

Smell	Taste	Texture	Color	Appearance	Treatment / days
1.672[d]	1.353[d]	1.311[d]	1.413[d]	1.330[d]	**15**
3.990[c]	3.640[c]	3.720[c]	3.832[c]	6.630[c]	**30**
4.512[b]	4.371[b]	4.261[b]	4.401[b]	4.313[b]	**45**
4.834[a]	4.673[a]	4.640[a]	4.771[a]	4.752[a]	**60**

Figures 2-5 were shown, the effect of different containers on sensory properties (appearance, color, texture, smell, taste) of smoked kutum fish. Different layers were separated in different color , layers 1 ($PET_{(12)}/AL_{(12)}/LLD_{(100)}$){blue}; layers 2($PET_{(12)}/AL_{(7)}/LLD_{(100)}$) {red}; layers 3 ($PET_{(12)}/AL_{(7)}/PET_{(12)}/LLD_{(100)}$) { green }.According to figure 2 , effect of different multilayer films was observed after 15 days on sensory evaluation, The lowest mark of sensory properties belonged to layer : 3 (4-layers) and then layer:1(3-layers).

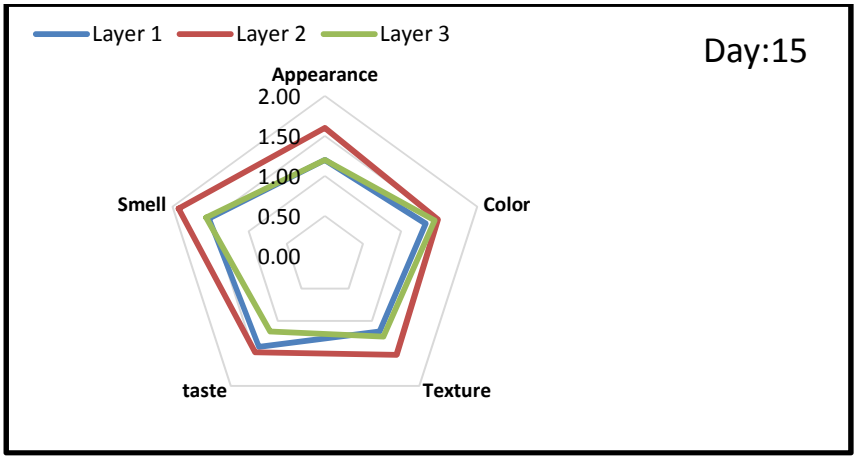

Figure 2: Effect of different layers after 15 days on sensory evaluation

According to figure 3, the effect of different containers on sensory evaluation (appearance, color, texture, smell, taste) of smoked kutum fish after 30 days was shown. The lowest mark of sensory properties belonged to layer: 3(4-layers) and then layer:1(3-layers).

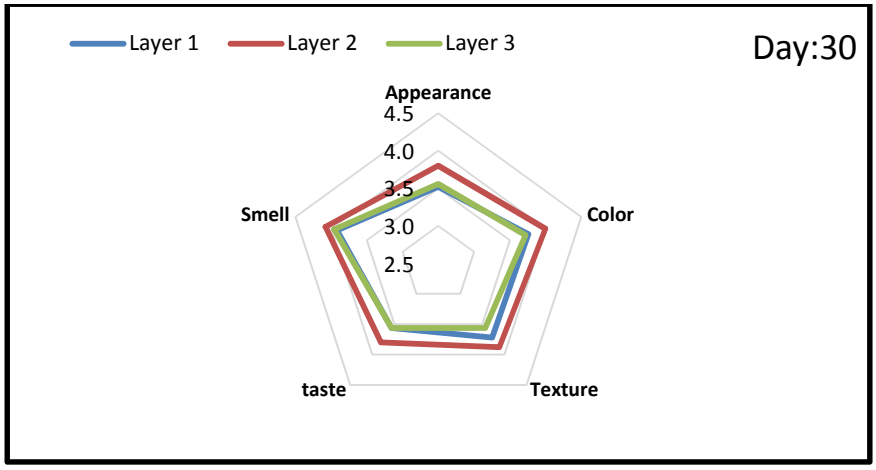

Figure 3: Effect of different layers after 30 days on sensory evaluation

According to figure 4, the effect of different containers on sensory evaluation (appearance, color, texture, smell, taste) of smoked kutum fish was observed after 45 days. The lowest mark of sensory properties belonged to layer: 3(4-layers) and then layer:1(3-layers).

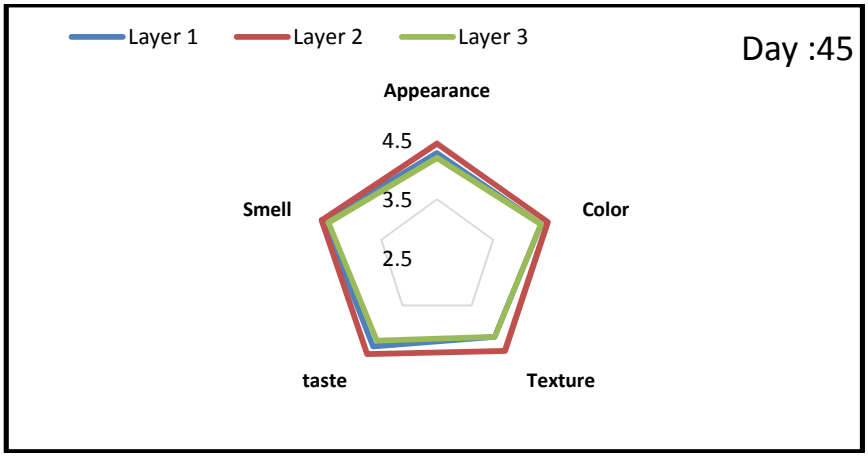

Figure 4: Effect of different layers after 45 days on sensory evaluation

According to figure 5, the effect of different containers on sensory evaluation (appearance, color, texture, Smell, taste) of smoked kutum fish was shown after 60 days. The lowest mark of sensory properties belonged to layer: 3(4-layers) and then layer:1(3-layers),but highest mark was for layer: 2(3layers).

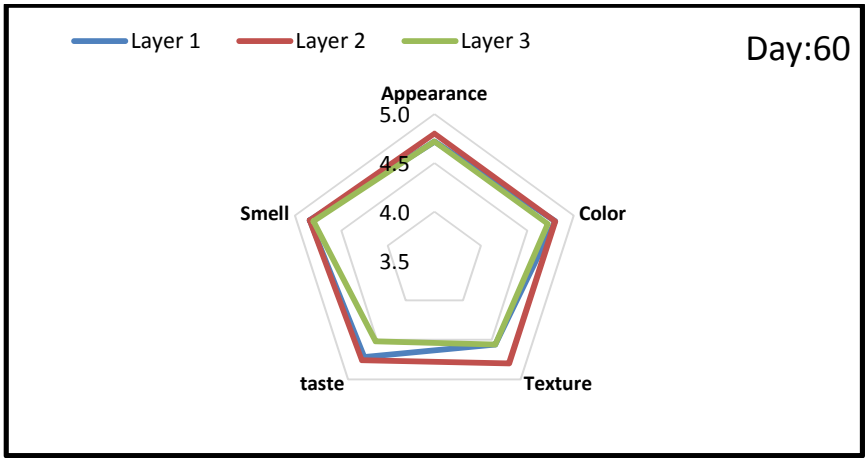

Figure 5: Effect of different layers after 60 days on sensory evaluation

Figures 6 -9 were shown, the effect of different gas compositions on sensory properties of smoked kutum fish. Different gas composition were separated in different color, (1- CO2 70% +N2 30%) {dark blue}; 2- CO2 30% +N2 70% {red}; 3 - CO2 45% +N2 45%+ 10% O2 {green}; 4-vacuum {Violet}; 5-control sample {blue}). According to figures 6, the effect combination of different gases after15 days on sensory evaluation was shown. The lowest mark of sensory properties belonged to gases combination 1 (70% CO2 + 30% N2) and then condition 3 (45% CO2 + 45% N 2+10%O2), highest mark observed in ordinary and vacuum conditions.

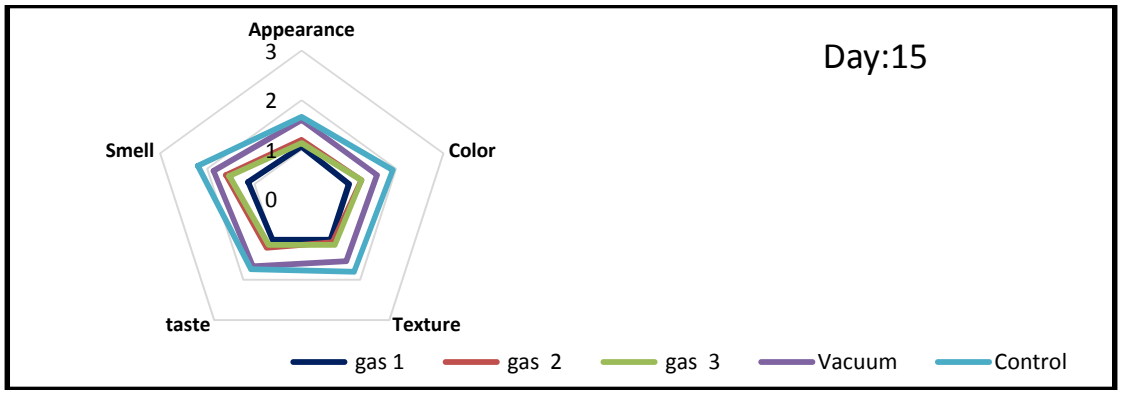

Figure 6: Effect of different gas combinations after 15 days on sensory evaluation

According to figure 7, the effect of different gas compositions were observed after 30 days on sensory evaluation. The lowest mark of sensory properties belonged to gases combination 1 (70% CO2 + 30% N2), and then condition 3(45% CO_2 + 45% N2+10%O2) ,highest mark observed in ordinary and vacuum conditions.

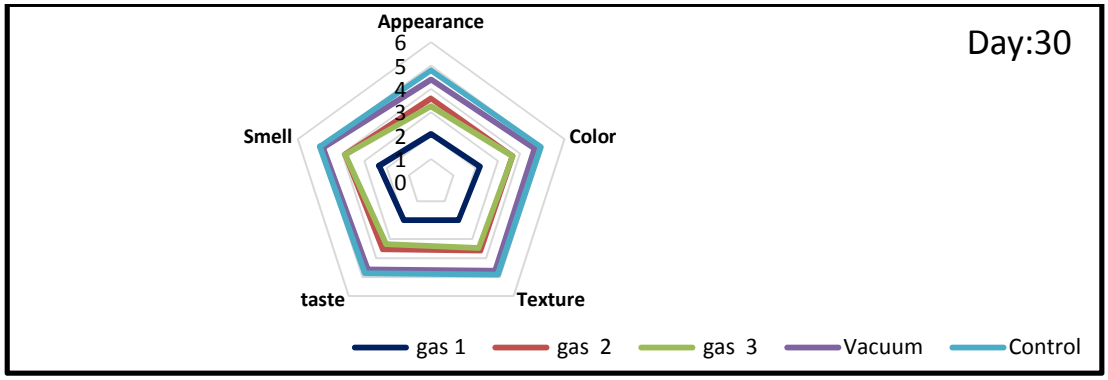

Figure 7: Effect of different gas combinations after 30 days on sensory evaluation

According to figure 8,the effect of different gas compositions were observed after 45 days on sensory evaluation. The lowest mark of sensory properties**(**appearance, color , texture **,** Smell**,** taste**)** belonged to gas combination 1 (70% CO_2 + 30% N2),and then condition 3(45% CO_2 + 45% N2+10%O2) , highest marked showed in ordinary and vacuum condition.

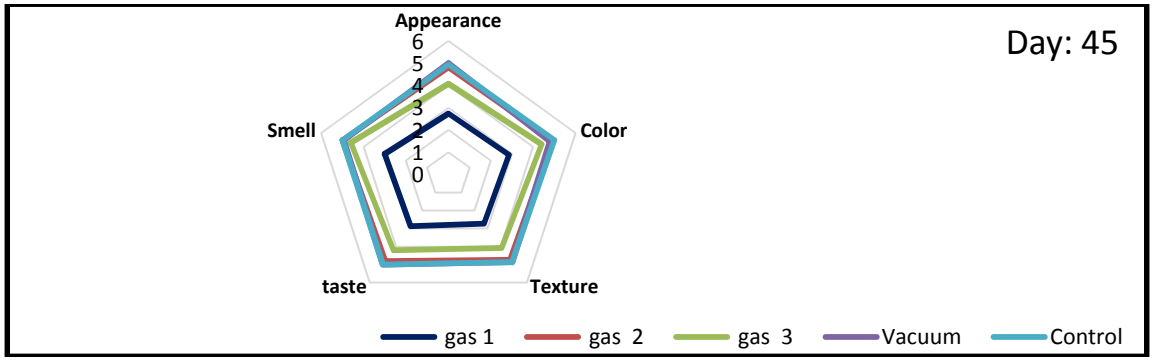

Figure 8: Effect of different gas combinations after 45 days on sensory evaluation

According to figure 9, the effect of different gas combinations was shown after 60 days on sensory evaluation. The lowest mark of sensory properties belonged to gas combination 1 (70% CO_2 + 30% N2), highest mark observed in ordinary conditions.

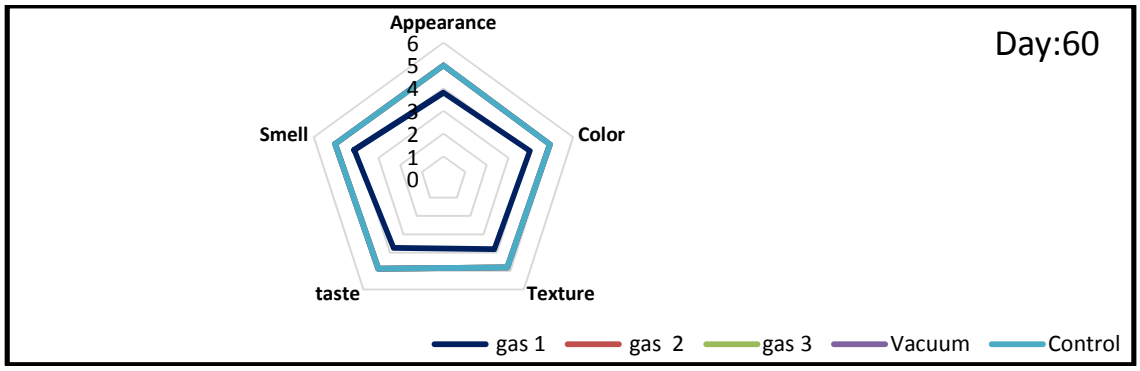

Figure 9: Effect of different gas combinations after 60 days on sensory evaluation

DISCUSSION

The best condition belonged to samples in container 3: 4- layer under conditions (70% Co_2) which were prolonged the shelf life of smoked kutum fish till 60 days. Protection of these samples in 4-layers was better than 3-layers(AL:12) and 3-layers (AL:7), because of steam permeability of 4-layers was lower than 3-layers ,so the usage of this container was better for preserving smoked kutum fish in long time. Especially taste & texture had better result in this technique.

Vanderzant *et al.*, 2000 , due to Centralized packaging of beef lion steaks with different oxygen-barrier films under vacuum and MAP condition during 30 days were seen significant difference with ordinary condition and the results were corresponded with this investigation. Erkan *et al.*,2006, indicated that due to effet of Spoilage and shelf life of sardines (*Sardina pilchardus*) packed in modified atmosphere, results were similar to these results. Chouliara & Karatapanis, 2007 , due to research about combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat during 11 days , the results of sensory evaluation were similar to these results. Athina *et al*., 2008, due to research about microbial flora and sensory changes in smoked turkey breast fillets stored under various packaging conditions at at 4°C, the results of sensory evaluation were corresponded with this investigation.

Zand *et al*. ,2010, indicated that due to shelf life extension of cooked chick and chick meal in multilayer flexible pouches 4-layer container was better than 3-layer, results were corresponded with this study. Zand & Mailova ,2010, due to research about the shelf life prolongation of packed meals in multilayer flexible pouches conducted 4-layer container was better than 3-layer, for preservation sensory properties, the results were similar to these results. Zand & Sotoudeh ,2013, indicated that due to the influence of MAP in multilayer flexible pouches on sensory properties of chicken meal,the best result belonged to 4-layer under CO2 %70+N 2 %30, that the results were corresponded with these results. Zand & Allahyari, 2013, due to the influence of MAP and different multilayer flexible films on sensory evaluation of candy bread in 3 week, the results under CO2 %70 in 4-layer container, were corresponded with these results. Zand, 2013,conducted 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 sensory evaluation, were corresponded with these results. 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 sensory evaluation, were similar to this study.

CONCLUSION

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 microorganism without a significant adverse effect on food properties and controlled sensory properties of fish samples as well as possible. In the present study, it was concluded that, sensory properties and shelf life of packed smoked kutum fish (*Rutilus Frisii Kutum)* have been affected by different flexible multi-layer containers and different concentrations of three gas mixture (carbon dioxide, nitrogen, oxygen), and also vacuum conditions during 60 days. These parameters could be promoted, substitution of MAP and these barrier containers instead of traditional packaging in food industries, due to a lot of privilege of them for shelf life extension of seafood in long times.

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