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Effect of modified atmosphere packaging and multi-layer flexible films on pH 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 on pH of smoked kutum fish (*Rutilus frisii kutum*) at ambient condition (T = 25 ˚C) were evaluated. Ordinary conditions as control packaging were compared with four types of modified atmosphere packaging: (70% N_2 + 30% CO_2), (30% N_2 + 70% CO_2), (45% CO_2 + 45% N_2 + 10% O_2), and vacuum conditions. Smoked Kutum fishes were packaged in flexible multi-layer films under modified atmosphere packaging, 3-layer {($PET_{(12)}/AL_{(12)}/LLD_{(100)}$), 4-layer ($PET_{(12)}/AL_{(7)}/PET_{(12)}/LLD_{(100)}$) and 3-layer ($PET_{(12)}/AL_{(7)}/LLD_{(100)}$)}. Samples were performed chemical test (pH) at different times during 60 days ,with 15 treatment ,3 run, statistical analysis and comparison of data, were done by software SAS (Ver:9/1) and also Duncan's new multiple range test, with confidence level of 95% (P<0.05). The shelf life of smoked kutum fishes (according to pH changes) were reported under conditions 1, 2 and 3 in 4-layer, 60, 58 and 45 days and in vacuum conditions were about 40 days, in 3-layer (AL:12), under conditions 1, 2 and 3 and vacuum conditions were 55, 50, 40 and 35 days, with 3 layers (AL:7), under conditions 1, 2 and 3 were 45, 40 and 35 days and in vacuum conditions were 30 days. Maximum change of pH was related to 3-layer (AL:7) under 30% CO_2 + 70% N_2 and ordinary condition, the lowest changes belonged to 4-layer under 70% CO_2 + 30% N_2. The pH variable has not decreased rapidly, in each treatments during storage times (60 days), while can be explained by characteristic of this multi-layer flexible pouch with less water vapor and oxygen permeability, and increasing CO_2 caused to control some chemical reactions such as pH changes in samples.

Keywords: modified atmosphere packaging (MAP), flexible multi-layer films, smoked kutum fish (*Rutilus frisii kutum)*, pH

Introduction

It is well known that packaging performs many complementary functions; protecting the product against external polluting agents, giving the product marketing value and providing the customer with information about ingredients or how to use the product (Zand *et al*., 2010 a-b; Zand, 2013 a-b). As a result, packaging has become an indispensable element in the food manufacturing process. In order to meet the huge demand of the food industry, there has been a remarkable growth in the development of food packaging in the past decades (Zand *et al.,* 2010 a-b; Zand, 2013 a-b).Modified atmosphere packaging (MAP) has led the evolution of fresh and minimally processed food preservation. MAP refers to a condition initially produced at the time of packaging (Mortazavi *et al*., 2002; Athina *et al*., 2010). The gases within the package are allowed to change as the physical and biological conditions. Rather than preserving food through the extremes of heat (sterilization) or cold (freezing), MAP utilizes 'minimal processing' to preserve food with the absolute least amount of damage to quality, texture, taste and nutrition (Blackistone, 1998; Bingol and Ergun 2011; Sotoudeh *et al.,* 2013; Zand and Sotoudeh, 2013 a-b; Zand and Allahyari, 2013 a-b). Modified atmosphere packaging (MAP) has gained considerable popularity over the last decades as a modern non-thermal method of food preservation.

The proper combination of gases (carbon dioxide, nitrogen and oxygen) in the headspace of food packs results in suppression of the microbial flora of perishable food such as meat and fishery products have been developed under aerobic conditions for preservation of their sensory properties (Daniels *et al*., 1985 ; Erkan *et al.,* 2006; Chouliara and Karata-panis, 2007; Sotoudeh *et al.,* 2013; Zand and Sotoudeh , 2013 a-b; Zand and Allahyari, 2013 a-b).Modified atmosphere packaging is an enclosure of food , in a container 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

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activity. This modification is performed by gas flash packaging which oxygen is removed and replaced by a controlled mixture of gases (Vanderzant, *et al.*, 2000; Taylor, 2008). Although, thermal treatment (120 ˚C and 20 min) effectively destroys these microorganisms (Mortazavi *et al.*, 2002; Jay *et al.*, 2003), 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 (Cameron *etal*., 1989; Blackistone, 1998; Sotoudeh *et al.,*2013; Zand and Sotoudeh, 2013 a-b; Zand and Allahyari, 2013 a-b). Other hands such treatment is carried out at high temperature at which shrinkages and leakages of pouches have been occurred that caused second contamination (Mortazavi *et al.*, 2002; Jay *et al.*, 2003). For that reason, significant efforts are leading to the development of novel processing such as MAP (Sivertsvik *et al*., 2003; Sotoudeh *et al.,* 2013; Zand and Sotoudeh, 2013 a-b; Zand and Allahyari, 2013 a-b), which is proving to be able to inactivate spoilage microorganisms without significantly affect nutritional properties of several foods (Bingol and Ergun 2011).

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 gases $CO_2/N_2/O_2$ 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 (Cameron *et al*., 1989; Erkan *et al.,* 2006; Vanderzant *et al.*, 2000), 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 (Chouliara and Karatapanis, 2007; Taylor, 2008). Nitrogen (N_2) is a non-reactive gas that has no smell or taste, unlike carbon dioxide, is not absorbed in food or water (Cameron *et al*., 1989; Blackistone, 1998; Zand and Sotoudeh, 2013 a-b; Zand and Allahyari, 2013 a-b). It is used as a filler gas to replace oxygen and thus prevent spoilage or to replace carbon dioxide and prevent package collapse. Oxygen (O_2) prevents growth of anaerobic bacteria (Chouliara and Karatapanis, 2007; Bingol and Ergun 2011; Zand and Sotoudeh, 2013 a-b; Zand and Allahyari, 2013 a-b). The multi-layer films have been used for packaging in this project is polymers or plastic films laminated with aluminum instead of can (Paine and Paine, 1992; Sotoudeh *et al.,* 2013; Zand and Sotoudeh, 2013a-b; Zand and Allahyari, 2013a-b). 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 terephthalate (PET), which are barrier films (O'Meara *et al.*, 1977; Paine and Paine, 1992; Sotoudeh *et al.,* 2013; Zand and Sotoudeh, 2013 a-b ; Zand and Allahyari, 2013 a-b),and metallic components present in a package, such as aluminum foil, can dramatically influence on heating rates of the packaged food (Zand, 2011a-c; Sotoudeh *et al.,*2013; Zand and Sotoudeh , 2013a,b; Zand and Allahyari, 2013a,b).Kutum fish with the scientific name*, Rutilus frisii kutum*, it belongs to the Cyprinids family and has a high commercial value, The main distribution of this species from Turkmenistan to Azerbaijan along the Caspian Sea. It is one of the economically important fish in the region (Cai *et al.*, 1997; Hosseini *et al.*, 2012; ISIRI, 2013a).

The main purposes of this study are: A) investigation about the effects of modified atmosphere packaging; with different concentrations of $CO_2/N_2/O_2$ and B) usage of three multilayer flexible pouches (3 and 4-layer) (Sivertsvik *et al*., 2003; Zand, 2011a-c) for controlling pH of smoked kutum fish (O'Meara *et al.*, 1977; Paine and Paine, 1992; Chouliara and Karatapanis, 2007; Sotoudeh *et al.,* 2013; Zand and Sotoudeh, 2013a-b; Zand and Allahyari, 2013a-b). We want to prove MAP can substitute thermal processing in conservation industries, and have a lot of privilege for shelf life prolongation of seafood and control pH variable of these products (Zand, 2011a-c; Sotoudeh *et al.,* 2013; Zand and Sotoudeh, 2013a-b; Zand and Allahyari, 2013a-b).

Materials and Methods

Preparation of smoked kutum fish

Five 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 experiment. The head and tail of samples, were isolated and then samples of fish, divided into small pieces (60 g) and placed under sterile conditions inside the containers. Temperature was controlled in order to decrease to ambient temperature (T = 25°C). Samples of smoked fishes were ready for packaging and gas injection. 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 (Zand 2011a-c; Sotoudeh *et al.,*2013; Zand and Sotoudeh, 2013a-b; Zand and Allahyari, 2013a-b).

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 different gas composition {(%30 N_2 + %70 CO_2), (%70 N_2 + %30 CO_2), (45% CO_2 + 45% N_2 + 10% O_2)} and vacuum condition by modified atmosphere packaging machine. After packaging, samples were put in ambient condition (room temperature), for determination chemical test (pH) (Sotoudeh *et al.,* 2013; Zand and Sotoudeh, 2013a-b; Zand and Allahyari, 2013a-b).

Chemical tests

Measurements: pH

PH meter was adjusted with a buffer 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) (ISIRI, 2003a; ISIRI, 2003b; Zand, 2011a-c; Sotoudeh *et al.,* 2013; Zand and Sotoudeh, 2013a-b; Zand and Allahyari, 2013a-b).

Samples packaging and storage

All pouches (smoked kutum fish), were put in at ambient condition (T= 25°C).Samples were packaged into three multilayer flexible films. Analytical characteristics of these barrier containers were shown in Table 1 (Zand, 2011a-c; Sotoudeh *et al.,* 2013; Zand and Sotoudeh, 2013a-b; Zand and Allahyari, 2013a-b).

Tab. 1: Analytical characteristics of containers. (Zand, 2011a-c; Sotoudeh *et al.,* 2013; Zand and Sotoudeh, 2013a,b ; Zand and Allahyari , 2013a,b).

Sample	Layers	Thickness (µ)	Tensile of sealing film (N)	O.T.R ml/m².dav	W.V.T.R g/m².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 smoked kutum fish, and type of treatments on pH during different storage times (15, 30, 45 and 60 days). Statistical analysis of data, was performed by software Statistical Analysis System (SAS 9/1) with ANOVA test, and comparison of data was done by Duncan's new multiple range test, with confidence level of 95% (P<0.05) (Zand, 2011a-c; 2013a-b; Zand and Allahyari, 2013a-b).

Results

Effect of MAP on pH of smoked kutum fish

Analysis of variance (Tab. 2) showed that, the effects of different storage times (days), different gas compositions and different layers on pH had significant difference (P<0.01). The interaction of (gas × time) had significant difference (P<0.01). The variance of pH was depended on amount of CO_2 in packages which were absorbed by the samples. The decreased of pH can be explained by surface reaction of CO_2 with water and forming carbonic acid. Smoked kutum fish were packaged in 4-layer ($PET_{(12)}/AL_{(7)}/PET_{(12)}/LLD_{(100)}$) under (70% CO_2 + 30% N_2) conditions with the lowest CO_2 transmission rate and retained more CO_2 within packages, had the lowest pH of smoked kutum fish.

Tab. 2: Analysis of variance mean squares traits in response to treatments.

pH	Degrees of freedom	Variables
0.155**	2	Layer
0.261**	4	Gas
0.0038**	8	Gas × Layer
0.0815**	3	Time (day)
0.0011ns	6	Layer × Time (day)
0.076**	12	Gas × Time (day)
0.0011**	24	Layer × Gas × Time (day)
0.0008	120	Errors
3.397	-	CV (Variance Index)

Table 3 and Figure 1 were shown the effect of different layers on pH of smoked kutum fish. Different layers were separated in different colors. In Figure 1, layer: 1 ($PET_{(12)}/AL_{(12)}/LLD_{(100)}$){blue}; layer: 2 ($PET_{(12)}/AL_{(7)}/LLD_{(100)}$){red}; layer: 3 ($PET_{(12)}/AL_{(7)}/PET_{(12)}/LLD_{(100)}$){green}. The lowest amount of pH belonged to 4-layer and the highest amount observed in 3-layer (AL:7), due to the thickness (131µ), and low permeability of water vapor in this container.

Tab. 3: The effect of different layers on pH.

pH	Different layers
6.17[b]	Film 1, 3-layer (AL:12)
6.23[a]	Film 2, 3-layer (AL:7)
6.13[c]	Film 3, 4-layer

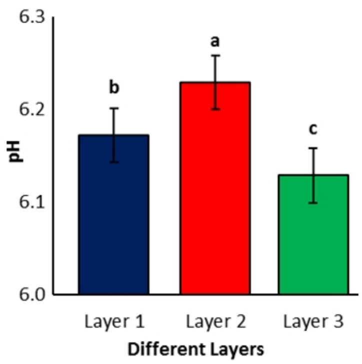

Fig. 1: The effect of different layers on pH.

Table 4 and Figure 2 were shown, the effect of different gas compositions on pH of smoked kutum fish. Different gas compositions were separated in different colors in Figure 2 , e- 70% CO_2, 30% N_2 {red}; b-30% CO_2, 70% N_2 {blue}; d- 45% CO_2, 45% N_2 {green}; d-Vacuum{pink}; a-control sample {black}.The changes pH will increase**.** According to Table 4 and Figure 2, the lowest amount of pH belonged to condition under (70% CO_2 + 30% N_2), and the highest amount observed under ordinary condition due to type of gas atmospheres. This mechanism could be described by its solution in water of food tissue and produced carbonic acid which the more carbonic acid was decreased pH more.

Tab. 4: The effect of different gas compositions on pH.

pH	Gas composition
6.09e	70% CO_2 + 30% N_2
6.24b	30% CO_2 + 70% N_2
6.15c	45% CO_2 + 5% N_2 + 10% O_2
6.11d	vacuum
6.29a	control

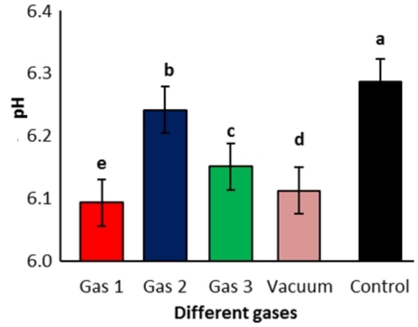

Fig. 2: The effect of different gas compositions on pH.

Table 5 and Figure 3 were shown, the effect of different times on pH of smoked kutum fish. Different days were separated in different colors in Figure 2, days15 {red}; days 30{blue}; days 45 {green} days60 {black}.The pH of samples increased**.** According to Table 5 and Figure 3, the lowest amount of pH was reported after 15 days and highest after 60 days, which caused bacteria to grow by the times and changed pH.

Tab. 5: The effect of different days on pH.

pH	various days
6.17[b]	Day 15
6.13[c]	Day 30
6.24[a]	Day 45
6.17[b]	Day 60

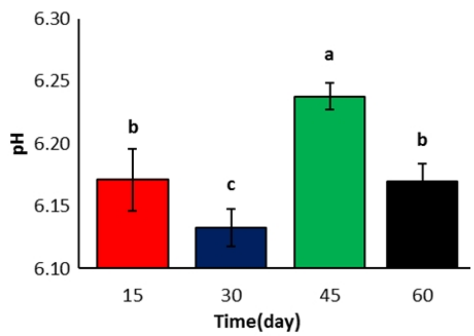

Fig 3: The effect of different days on pH.

According to Figure 4, the effect of different combination of gases and different storage times (60days) in layer: 1 ($PET_{(12)}/AL_{(12)}/LLD_{(100)}$) on pH of smoked kutum fish were shown. The lowest amount of pH of smoked kutum fish belonged to gas compositions, vacuum after 15 days, and condition 70% CO_2 + 30% N_2 after 30 days, highest amount belonged to condition 30% CO_2 + 70% N_2 after 15 days, then control condition after 60 days.

With regard to Figure 5, the effect of different combination of gases and different storage times (60 days) in layer: 2 ($PET_{(12)}/AL_{(7)}/LLD_{(100)}$) on pH of smoked kutum fish were shown. The lowest amount of smoked kutum fish belonged to gas compositions, 70% CO_2 after 30 and 70% CO_2 after 60 days too, highest amount belonged to condition 30% CO_2 + 70% N_2 after 15 days, then control condition after 45 and 60 days.

Based on Figure 6, the effect of different combination of gases and different storage times (60 days) in layer: 3 ($PET_{(12)}/AL_{(7)}/PET_{(12)}/LLD_{(100)}$) on pH of smoked kutum fish were shown. The lowest amount of smoked kutum fish belonged to vacuum after 15 days, then condition 70% CO_2 + 30% N_2 after 30 days, highest amount belonged to condition 30% CO_2 + 70% N_2 after 15 days, then control condition after 30 and 45 days.

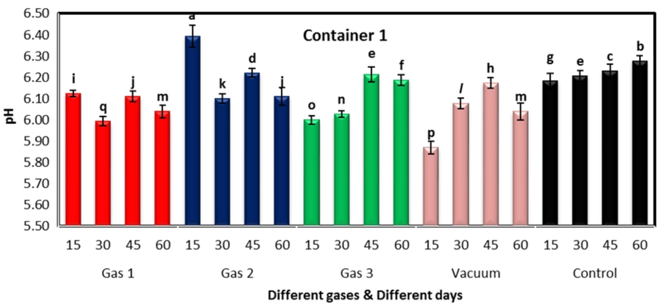

Fig 4: The effect of different gas compositions and different days on pH in container 1.

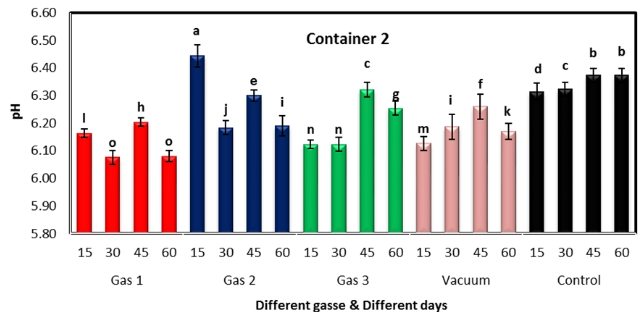

Fig 5: The effect of different gas compositions and different days on pH in container 2.

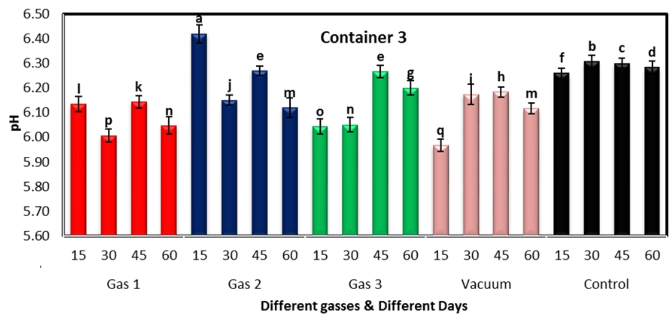

Fig. 6: The effect of different gas compositions and different days on pH in container 3.

Discussion

The pH variable had not decreased rapidly, in each treatments during storage times, while could be explained by characteristic of this multi- layer flexible pouches with less water vapor and oxygen permeability, and usage of modified atmosphere ,caused to control chemical reactions such as pH in different conditions. The shelf life of smoked kutum fish (according to pH changes) were reported under conditions 1, 2 and 3 in 4-layer, 60, 58 and 45 days and in vacuum conditions were about 40 days, in 3-layer (AL:12) , under conditions 1, 2 and 3 and vacuum conditions were 55, 50, 40 and 35 days, with 3-layer (AL:7),under conditions 1, 2 and 3 were 45, 40 and 35 days and in vacuum conditions were 30 days.Maximum changes of pH were related to 3-layer (AL:7) under CO_2 30% + N_2 70% and ordinary condition too, the lowest pH changes belonged to 4-layer under 70% CO_2,which were controlled pH of fish samples till 60 days. Changes of pH in various conditions, had significant differences between (layers, gas) and (layer and time) and also (gas and time) ($P<0.01$).

Vanderzant *et al*., (2000) indicated that based on centralized packaging of beef steaks with different oxygen-barrier films under vacuum and MAP condition during 30 days were observed significant difference with ordinary condition, and the results were corresponded with these results. Taloyor (2008) showed effect of packaging fresh meat and poultry on pH under, concluded MAP conditions with high amount CO_2 was better than vacuum for pH more than 6 , that the results were similar to this investigation and best condition belonged to CO_2 70%. Zand *et al*. (2010 a-b) indicated that shelf life prolongation of cooked chick and chick meal in multilayer flexible pouches , 4-layer container was better than 3-layer, and the results on pH changes were corresponded with these result. Bingol and Ergun (2011) conducted the effect of two different types of gas carbon dioxide and oxygen had been performed on ostrich meat, the results showed that the shelf life of ostrich meat under %60 CO_2 condition prolonged till 7 days, that the results were similar to these results. The effect of packaging with multilayer flexible pouches on shelf life of chicken meal, changes of pH in 3-layer and 4-layer from day 0 to day 7 of preservation were increased and from day 7 till day 15 were reduced. But the lowest change of pH belonged to gas combination (30% N_2 + 70% CO_2) in 4-layer flexible films (131 μ) ,and the highest seen in ordinary condition in 3-layer flexible films (119 μ), that the results were similar to this investigation (Zand and Sotoudeh, 2013a-b). 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 and best gas combination belonged to 70% CO_2, and the results of pH changes were corresponded with these result (Sotoudeh *et al*., 2013). Due to researched about effect of packaging under gas combination (30% N_2 + 70% CO_2) in 4-layer flexible films on shelf life and pH of candy bread ,this container was better than two type of 3-layer flexible films (119 μ and 124 μ), that the results were similar to this investigation (Zand and Allahyari, 2013a-b). Zand and Sotoudeh (2013a-b) conducted the effect of packaging under gas combination (30% N_2 + 70% CO_2) in 4-layer flexible films (131 μ), was better than 3-layer flexible films (124 μ) on shelf life and pH of chicken meal, and the results were similar to this investigation. Zand (2013a) indicated that the shelf life extension of mushroom meal in multilayer flexible pouches 4-layer container was better than 3-layer during 60 days, the results on changes of pH were corresponded with these results. Zand (2013b) conducted 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,the results on changes of pH were similar to these results.

Conclusion

In the present study, it was concluded that, chemical reaction 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. 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 chemical reactions such as pH changes. 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 for shelf life prolongation of seafood.

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