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ORIGINAL ARTICLE

Increasing the time of Stability of common frozen kilka fish (*Clupeonella cultriventris*) by using Natural Antioxidant of extract of *Origanum valgare*

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ABSTRACT
Current research was done for evaluating the effect of extract of the plant of Origanum vulgare on quality and stability of the common kilka fish (Clupeonella cultriventris) in preservation condition as frozen for 4 months. For doing this research, two different treatments were designed each by 3 repeats. Fishes after cutting head and emptying belly were washed. Fishes of control treatments (lack of antioxidant) were immersed in distilled water for 10 minutes and fishes of treatment 1(having antioxidant) were immersed in 50ppm extract of Origanum vulgare for 10 minutes. Then fishes of both treatments were packed and kept in -18^{0}C for 4 months. Amount of fat, protein PH, TBA and TVB-N were assessed. The result of this research showed that using Origanum vulgare due to having antioxidant and antimicrobial feature, cause delay of oxidation spoiling and therefore increases stability of kilka (Clupeonella cultriventris) in frozen condition of preservation.
Key words: kilka(Clupeonella cultriventris), freezing, Origanum vulgare , TBA, TVB-N

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INTRODUCTION

Increasing population on the one hand and deficiency of protein resources and low price of kilka fishes comparing to other protein resources justifies the necessity of planning for enter of these species of fishes on the table food of consumers. Nowadays one of main reasons of deficiency of consuming aquatics in the country comparing to other countries is lack of variety in sea products and packing aquatics. Regarding increasing need of the society to feeding aquatic and planning for increase of consumption of aquatics in economic social development plan fish is a valuable food and if it is not preserved correctly in dominant catching it constitutes 53/87 percent of the whole catching it will be corrupted easily [1].

Main part of Anchovy kilka spawns in autumn until beginning of winter and in Nov it has the maximum spawning. Based on above studies biomass of Anchovy fish averagely during 4 seasons was estimated 212 thousand tons that 62/6 thousand tones exist under coastline(20-200m) and 149/7 thousand tones exist under deep region(more than 200m) [2-3].

Fisheries of Iran regarding evaluations developed navy of fishing vessels at Northern provinces as it develops from 94 ones in 1997 to 199 ones in 2002. With increasing process of fishing navy total degree of fishing in 1997 from 60thousand tones increased to 95 thousands tones [4].

One of main changes that happen during processing, distribution, storing and preparing food is oxidation of oils and fats that this event not only causes losing food quality but also produces oxidized products like free radicals that leads to the various unfavorable chemical reactions [5].

Application of antioxidant in food is one of the most effective methods of slowing oxidation of lipids and increasing life of preserving lipid food so it is preventing decrease of sensory and nutritional quality of them. Now antioxidant of Butyl hydroxy Anisole (BHA), Butylated hydroxytoluene(BHT), propil Galat (PG) and Tert-Butyl hydroquinone (TBHQ) have the highest expansion but consuming them due to

feature of anti-cancer and increasing desire of people for avoiding consumption or minimizing application of synthesis additives in food has been reduced. Therefore natural antioxidant like Tocopherol and derivatives of ascorbic acid entitled as secure natural antioxidant has been used extensively. Also observations denote that diets filled with herbal antioxidant endanger health of human in the view of making cancer and heart coronary heart diseases [7-10].

Consuming antioxidant in food industries are usually chemical types that in this experiment natural antioxidant of extract *Origanum valgare* is used.

The goal of doing this research is producing new products with favor of natural antioxidant with herbal origin.

MATERIALS AND METHODS

Common kilka fish (*Clupeonella cultriventris)* of Caspian sea was caught in March and transferred to the mixture with water and ice with tank of ACW to the center of science and technology park and firstly their heads were cut and intestine and offal became empty.

Then fishes were kept in cold water and after taking plasma they were floated in solution of the extract of *Origanum valgare* for 10 minutes at the temperature of 18 centigrade degree and then were exited and covered in disposable container by poly ethylene coverage sample lacking antioxidant was kept in distilled water as control and then packed and transferred into freezer with -18^0C.

Experiments include measuring pH and Thiobarbituric acid and amount of free fatty acids and proteins, were doe periodically every month for 4 month of preserving. It is necessary to mention that treatment of fish immersed by distilled water like above methods was considered as control treatment. In doing chemical experiment for total degree volatile nitrogenous base (TVB-N) from methods of Jeon *et al* [11], measurement indices of free fatty acids and Thiobarbituric acid (TBA) from method of Vyncke [12] , and pH determined by pH meter (Meter Lab pH M 93, Radiometer Analytical, Denmark).

Statistical analysis of data was done by SPSS software version 17.

RESULT AND DISCUSSION

Changes of amount of PH: figure 1 didn't show significantly difference at the degree of pH in treatment lacking of *Origanum valgare* during months of preserving fish in freezer but in treatment with *Origanum valgare* it dropped only at the third month and got meaningful difference with first and second month.

Generally changing range of PH was little and was a little more than 6 in first month and near to 7 at fourth month of sampling.

PH of muscles of fish is near to 7 but after death based on season, species and other factors it changes significantly [6].

Most fishes have low amount of carbohydrate (less than 0.5%) in their muscle tissue as after fish's death the amount of lactic acid produced due to reaction of glycolysis reduces and PH of meat of fish freezing of body will reach higher than 6 [13].

pH of fish's flesh has important effect on its freshness because its PH affects growth of bacteria. Usually due to increase of pH related to production of base like Ammonia, Trimethylamine and also other biogenic amines by bacteria is the reason of fish spoiling [14] and also it can be as the result of destruction and dissolution of proteins, peptides and amino acids available in fish muscles [15].

In this research in treatment having *Origanum vulgare* by spending time we observed reduction of pH not increase as in third and fourth months the least PH was observed that this action can have relationship with the antibacterial feature of *Origanum valgare*. Although PH of treatment lacking *Origanum valgare* was less that treatment having *Origanum valgare* but this action can be related to the primary PH of these two products that in treatment having *Origanum valgare* was significantly higher than treatment lacking it not to the improper effect of *Origanum valgare* at increase of bacterial activity or destruction of protein and finally increase of pH.

Reduction of pH during preservation period can be related to the solution of carbon dioxide (obtained from glycogen analysis) in water phase of muscles and therefore conformation of carbonic acid [16]. Increase of CO2 and therefore reduction of PH can prevent activity of bacteria and protein breaking and conformation of amines and on the one hand helps prevention of activity of internal protease [13] and certainly anti-microbial feature of *Origanum valgare* wasn't ineffective in this field.

Reduction of PH was observed in kilka fish treated with extract of thyme [17] that has shown similar result with current research.

Finally our hypothesis based on positive effect of extract *Origanum valgare* at increase of stability of kilka fish (*Clupeonella cultriventris)* in frozen preserving condition was proved and therefore achieved the goal

that was considered for increasing stability and preserving of protein quality of kilka fish (*Clupeonella cultriventris)* for linger period.

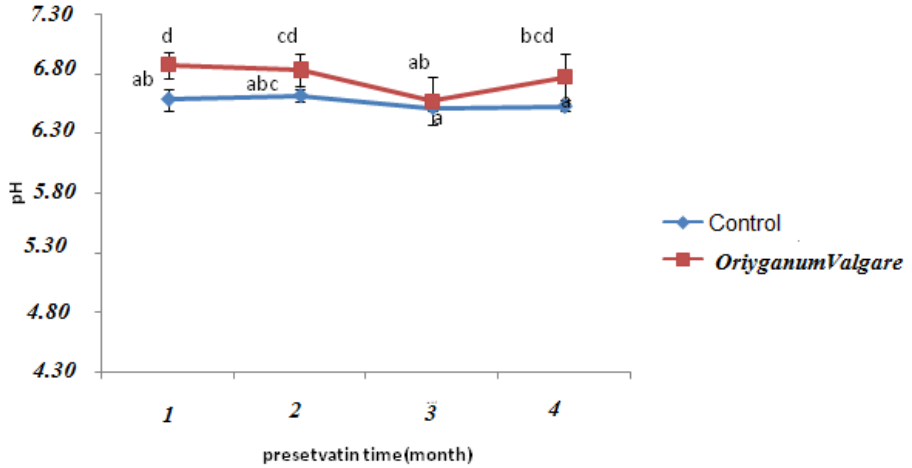

Fig 1: Changes of the degree of pH of different treatments during preservation time
Uncommon Latin letters shows meaningful difference between different treatments at the level of p<0.05 between treatments.

Changes of the degree of volatile nitrogenous base: The process of changes of TVB-N in figure 2 was increasing in both treatments but with the existence of increase of TVB-N over time, there hasn't been observed significant difference at its degree during second, third and fourth months in both treatments. Also although the degree of TVB-N in treatment having *Origanum valgare* was less than treatment lacking it, its degree in treatments during similar month of sampling didn't show significant difference.
Increase of the degree of TVB-N during preservation can be related to activities of bacteria producing spoiling . Relative low degree of TVB-N in treatment having *Origanum valgare* comparing to treatment lacking *Origanum valgare* may be due to anti-bacterial feature of *Origanum valgare* [18], that through inhibition of proteolic bacteria causing spoiling , prevents activity of these bacteria and prevents braking of proteins and therefore releasing nitrogenous compound.
Therefore in treatment having *Origanum valgare* in third month decrease of the degree of protein was accompanied by increase of the degree of TVB-N. In fact by spending more time the degree of activity and number of bacteria producing spoiling becomes more and therefore by breaking protein causes reduction of protein and increase of TVB-N.

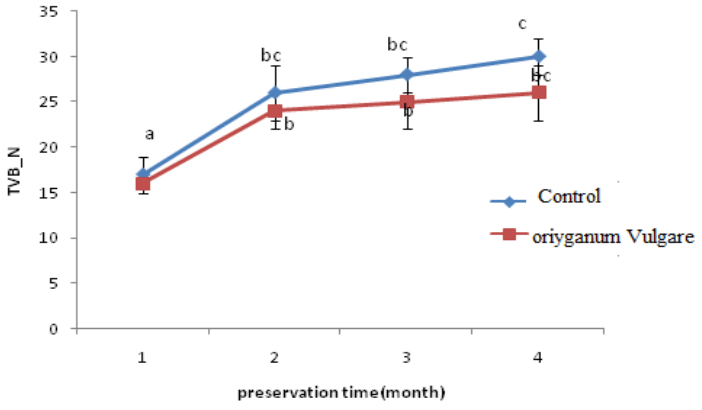

Figure 2 changes of the degree of TVB-N of different treatments during time of preservation
Uncommon Latin letters shows significant difference between different treatments at the level of p< 0.05 between treatments.

Changes of degree of TBA: Measuring TBA is proper index for determining improvement of fat oxidation and production of carbonyl compounds [10]. Existence of such compounds in fish meat cause changes in its sensory features such as flavor and smelling [19]. In fact index of TBA is related to the measurement of the degree of Malonaldehyde that is secondary product of oxidation of unsaturated multi-chain fatty acid [5].

In this research according to figure 3 the process of changes of TBA in both treatments was increasing process over time. As in treatment lacking *Origanum valgare* the degree of TBA in first, second and third month didn't have significant difference together but from third month it has increased significantly.

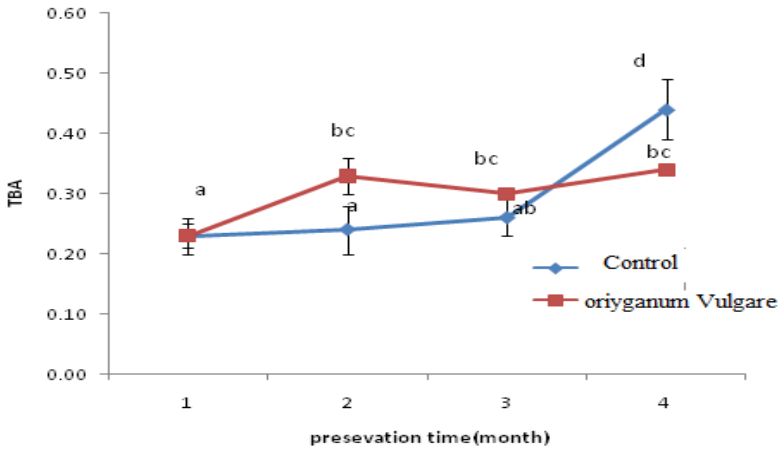

Figure 3-changes of the degree of TBA of different treatments during preservation time
Uncommon Latin letters show meaningful difference among different treatments at the level of P<0.05 among treatments.

Changes of Fat: Generally the degree of fluctuation of fat in treatment having *Origanum valgare* during 4 month didn't show meaningful difference between different month and significant difference was observed just between third and fourth months. In fact degree of fat of kilka having *Origanum valgare* at the fourth month has decreased significantly at thee forth month than third month but at the same level fat was observed at first and second month.
However degree of fat in kilka lack in *Origanum valgare* was relatively high as meaningful difference was observed at the degree of fat between months 1 and 3, 2 and 3, 3 and 4 and finally degree of fat at the fourth month reached to the degree of fat at the first month. Generally quality of frozen fish depends on hydrolyze and oxidation of its fat a lot.

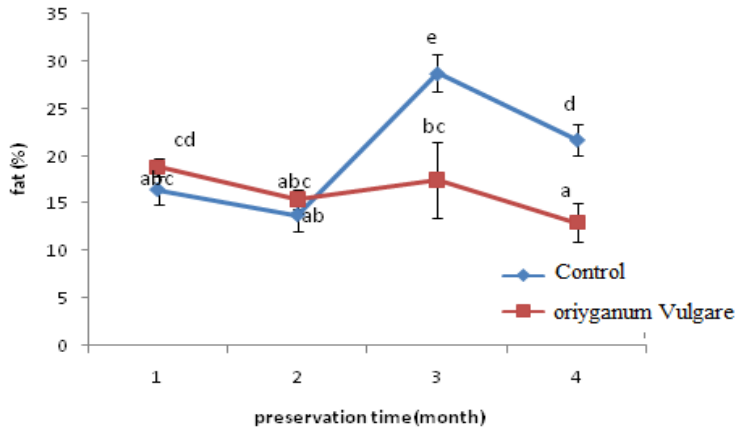

Figure 4-changes of the degree of fat at different treatments during preservation time
Uncommon Latin letters shows significant difference among different treatments at the level of P<0.05 between treatments.

Changes of the degree of protein: According to figure 5 the degree of protein of treatment lacking Origanum valgare passed increasing process as the least amount of protein in first month and the highest amount of protein was observed at third and fourth month that didn't have meaningful difference with each other. However, during treatment of *Origanum valgare* the degree of protein although increased at the second month and first month but at the third and fourth months decreased significantly than two first months.

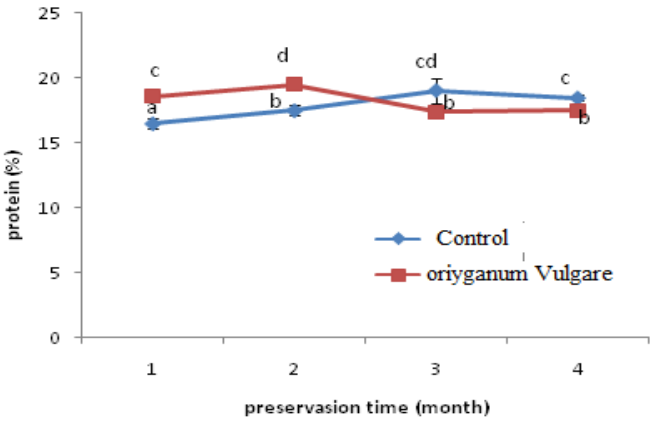

Figure 5-changes of the degree of protein at different treatments during preservation time
Uncommon Latin letters shows significant difference among different treatments at the level of $p<0.05$ among treatments.

CONCLUSION

Using *Origanum valgare* to the degree of 50ppm due to having the feature of antioxidant and antimicrobial, during preserving usual kilka (*Clupeonella cultriventris*) in frozen form causes delay of oxidation spoiling and therefore increase of stability of this fish.

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