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Research Article

Use of chitosan to shelf life extension of Siberian sturgeon (*Acipenser baeri*) caviar

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Abstract

This study was carried out to evaluate the feasibility of chitosan, a natural antimicrobial substance to improve the caviar shelf life. The effect of chitosan coating was carefully studied within 150 days storage time at -3°C in a standard situation. Both the caviar wrapped by chitosan and the control sample, were packed in commercial glass Jars. Chemical, microbiological and sensory analyses were done during storage time. Results showed that the effect of chitosan coating on caviar samples was to maintain their good quality characteristics and extend the shelf life of caviar significantly (p<0.05) about 60 days in contrast with standard sample.

Keywords: Chitosan, Caviar, Storage time, Shelf life, Packaging, *Acipenser baeri*

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Introdution

Caviar is a tasty and nourishing which comes in pasteurized and fresh (salt cured) form. The economic value of caviar is often reduced and its culinary gets affected by pasteurization. Caviar is produced from *Acipenser* fish specious eggs. Estimating the exact number of *Acipenser* species is one of the most controversial issues among scientists. Nevertheless, Scientists have been able to identify about twenty-seven species of Caviar in the Acipenser genus (Coad, 2003: Tavakoli et al.,2021).

Sturgeon is a common name for Acipenseridae family. Sturgeon has a record of near 300 million years. Scientists introduced the Sturgeon as a living fossil because it has been changed slightly during millennia. The sturgeon usually lives in coastal and fresh waters of some Asian and European countries such as Turkey, Iran, Azerbaijan, Bulgaria, and North America. Job opportunities and income generation are mainly important in the Caviar industry for these countries (Coad, 2003: Codex, 2010) .Chemical and bacterial spoilage of caviar is very high and this is due to its composition such as lipid contents, high protein (25%), presence of tissue materials and and most importantly the lack of pasteurization treatment during its processing (Salmani *et al.*, 2009; Anvar *et al.*, 2019; Haghighi *et al.*, 2022).

The role of Iran among caviar exporting countries is very notable and important. The average global caviar production (aquaculture and catch) was about 23000 MT per year (1950-1987). Russia's production was about 20000 MT during this period. Iran and the United States ranked second and third in the world by production of 1968 MT and 747 MT respectively. It is estimated that prevention of caviar spoilage and shelf life extension will be very vital for producing and exporting countries (Javhaudi and Sagheb, 1994).

Preservatives such as chitosan and nano-chitosan (Ghorabi and Khodanazary, 2020; Kamani *et al.*, 2020), silver/ copper/ titanium dioxide-nanocomposite (Kargar *et al.*, 2021), Nanosilver-TiO2 photocatalytic nanocomposite (Azari *et al.*, 2020), Date extracts (Seifzadeh and Rabbani Khorasgani, 2020) and *Capparis spinosa* root extract (Khademi *et al.*, 2020) reported to extend the shelf life of caviar or fish fillet. In general, naturally extracted food preservatives are much approved and required by consumers, unlike artificially produced ones. There is a wide spectrum of biomolecules, which induce antimicrobial properties in living organisms and take part in host defense processes. These molecules have very strange potential to be used in food industry as food preservatives. Lactoperoxidase, lysozyme, and chitosan are examples of natural preservatives, which exist in milk, egg white, and shrimp cells, respectively (Msagati, 2013).

Chitosan is a deacetylate form of chitin which naturally originated from crustaceans, insects and certain fungi. Chitosan has unique characteristics such as biodegradability, biocompatibility, and biological activity like antibacterial

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and anti-oxidative activity.it is widely used in cosmetics and food industry (Kumar, 2000; Fan *et al.*, 2009).

Chitosan discovery came back to the mid-18th century but its properties and application remained unknown until crystalline structure of chitosan were uncovered in 1934.Consequently, further studies about chitosan introduced it as safe and degradable preservatives in food industry. Economical and easily available to chitosan source such as mold and crustacean shells causes lead to be used in diverse application (Pillai, 2011).

The antimicrobial and functional activity of any preservatives are always affected by theirs component such as structure, method application, and nature of food products. Majority of researches are focused on development materials with coating technique and film forming capacity with antimicrobial properties which help to improve good quality and food shelf life extension of during the storage time. So, these kinds of films based on their function and application (viscosity, gel-forming ability, hardness, adhesiveness, thickening quality) could be used in variety of food stuffs (Kim, 2010: Ahari et al., 2022).

Kinds of films used as food preservatives and antimicrobials are not new concepts but the importance of using chitosan and other biofilms are very strong feedback of the consumer demands for nonchemical and recyclability food stuffs (Kim, 2010).

Materials and methods

Chitosan Preparation

Chitosan powder was obtained from SIGMA- ALDRICH (a part of Merck). The chitosan had a medium molecular weight with a deacetylation range of 75–85%. As a chitosan maker recipe first 2_{gr} chitosan powder and 100 ml Distilled water was mixed in a 250 ml decanter glass until the solution was homogenized then 2 ml acetic acid was added and mixed until the liquid was homogeneous and clear, which took around 0.5-1 hour (Xu *et al.*, 2005)

Sample preparation of caviar and chitosan

After removing the ovaries of fish, firstly ovarian membrane was isolated by special sieves and filtrated caviar was washed with cold water (10°C), then caviar rinsed and excess water was removed. Based on the weight of fish eggs, sodium chloride was added to the amount of 4% (according to initial tests), thoroughly mixed with caviar, and then rinsed and out of the excess brine. In the next step because of inducing chitosan, caviar which treated by salt mixed for 120 minutes with prepared chitosan suspensions in 2% acetic acid as a coated sample (Fan *et al.*, 2009). After this stage, additional suspension was removed and the resulting caviar filled in 50g cans. Air exhaust be done at this stage and cans were stored at -3^{o} ^{C} for 150 days. Microbiological, chemical and sensory factors of caviar samples were evaluated randomly every 30 days in the storage time.

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Chemical analyses

pH Determination

10_{gr} of caviar sample was homogenized in 100ml of distilled water and the mixture was filtered. The electrode of PH meter was dipped into the filtrated suspension and PH of caviar sample was measured by digital PH meter (Cyber scan PC 510 UK) (Fan *et al.*, 2009).

Determination of total volatile basic nitrogen (TVB-N)

As an Iranian national standards test methods and according to (Goulas and Kontominaz, 2005) 10 gr of caviar mixed with 50ml distilled water and transferred with 250mL distilled water into a 500ml round bottom flask and was distilled after addition of 2gr MgO, anti-foam and anti-bumping. On the other side 250ml Erlenmeyer flask containing 2%boric acid, methyl red and methylene blue was installed as the distillate receiver. distilling flask liquid was boiled about 30 minutes. The boric acid solution color was changed to green by alkaline distilled ammonia (TVB-N). This color changes titrated by 0.1N Hydrochloric acid. The quantity of TVBN in mg/100 g of Caviar was calculated from the hydrochloride volume(v) and its concentration(c) as follows:

TVB-N mg/100gr=(V*C*14*100)/100

Determination of peroxide value (PV)

Peroxide values of extracted oils were measured by titration of liberated iodine with standardized sodium thiosulphate solution in presence of 1.0% soluble starch as indicator, according to the AOAC official method (Association of official analytical chemists (AOAC, 1975).

Measurement of free fatty acids (FFA)

Oil extraction of caviar was done by solvent. Caviar sample (30gr) homogenized and mixed by blender with mixture of chloroform(30ml) and methanol(60ml) for 2minute.distilated water (30mL) was added to mixture for additional 30 seconds. The solution was stirred and filtered by Whatman No,1 filter paper. The lower clear phase was drained and concentrated by evaporating at 40°C (Bligh *et al.*, 1959). Free fatty acids, as oleic acid percentage in oil samples, were determined using an alkali titration method according to AOCS official Method according to AOCS Official Method (AOCS, 2017).

Determination of Thiobabituric acid value (TBA)

Thiobarbituric acid value was determined by the extraction method described by (Witte *et al.*, 1970). Practically 10_{gr} of caviar sample was grinded with 25 ml of Tricloroacetic acid 20 % (TCA) in 2M Ortophosphoric acid solution for 2 minute. The solution was filtered through Whatman paper to get TCA extract.3ml of TCA extract was mixed with 3ml of TBA reagent (0.005M) in test tubes and placed in a dark room for 16 hrs. A blank sample was prepared by mixing1.5ml of 20%TCA, 1.5ml distilled water and 3ml of 0.005M TBA reagent. Absorbance (O.D) was measured at fixed wavelength

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of 532nm whit a scanning range of 531nm to 533 nm using UV-VS spectrophotometer (Elico SL-159, Mumbia, India).

Microbiological analyses

Sample preparation of caviar was carried out according to ICMSF[1] (1986). Ten grams of caviar mixed with 90ml of sterile peptone water 1%. The mixture was homogenized about 3minut by sterile homogenizer. Total viable count (TVC) incubating was performed at 35°C for 48 ± 2 h and yeast and mold count at 25°C for 48±2 h. The samples were enumerated according to the American Public Health Association method (Spek, 1984).

Sensory analysis

The sensory analyses were performed by 14 tasters. They were selected as a caviar tester after sensory check and initial training. Comparison were carried out about sensory factors such as, texture, flavor and general acceptability. these factors evaluated by Hedonic test method which balanced by five point. This point arranged from one to five which characterized by supper bad to supper good respectively (Singh-Ackbarali and Maharaj, 2013).

Statistically analysis

The obtained data were statistically analyzed using SPSS (SPSS version22) to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to test for difference between means (significance was defined at $p<0.05$).

Results

Microbial Analyses

TVC of chitosan coated caviar sample was observed to be increasing more slowly than control sample and reached 5 log10 CFU/g on the 150th day of storage time, while the TVC of control sample reached about 5.4 log 10 CFU/gr on the 90th day and 5.8 log CFU/gr on the 150th day of the storage time. Changes in TVC of caviar sample during the storage time are shown in Figure 1.

Yeastand molds of control sample reached about 4.4 log10 CFU/gr on the 150th day while chitosan coated caviar reached to 4.5 log1o CFU/gr and 4.9 log10CFU/gr on the 90th and150th days of storage time respectively. Yeastand molds grow of two sample during storage time are shown in Figure 2.

Chemical Analysis

pH

Changes in value of PH during the storage time are shown in Figure 3. The initial PH reported about 6.0 in all samples of caviar. chitosan coated caviar PH was observed to be increasing more slowly than control sample and reached 6.45, 6.55, 6.85 on the 60th, 90th, and150th days of storage time respectively, while the PH of control sample reached about 6.77, 7.16, 7.21 on the same storage time.

[1] - International Commission on Microbiological Specifications for Food (ICMSF)

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Thiobarbituric Acid (TBA)

Increasing trends of Thiobarbituric acid (TBA) value of control sample such as other shelf-life indexes was very slower than the coated sample during the storage time. Results showed TBA value of control sample reached about 1.22, 1.6, 2.42 and 3,78 mg MDA/kg on the $30^{th,}$ 60^{th}, 90^{th}, and 150^{th} days of storage time respectively, while the chitosan coated caviar TBA value reached to 0.77, 1.19, 1.31 and 1.91 mg MDA/kg on the same storage time. Variation in TBA value are shown in Figure 4.

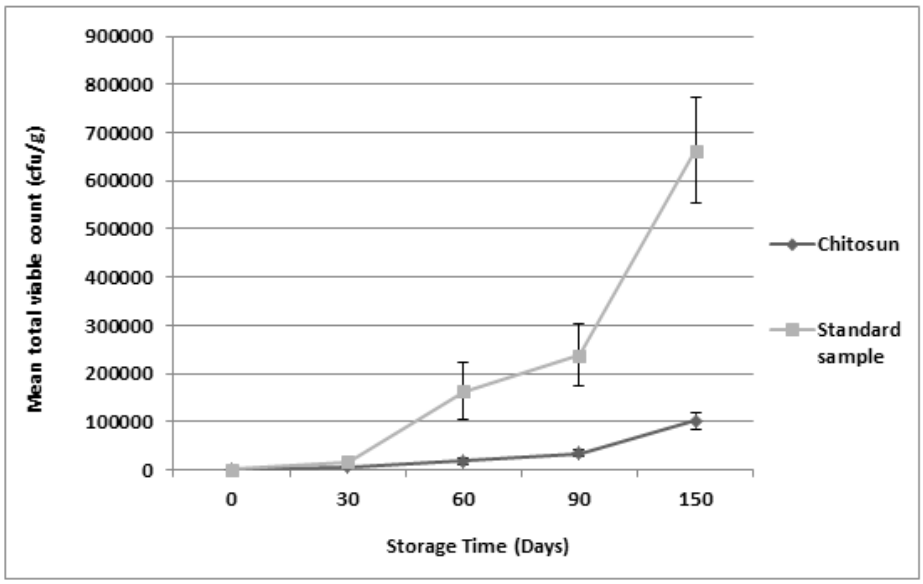


Figure 1: Changes in TVC of caviar sample during storage time.

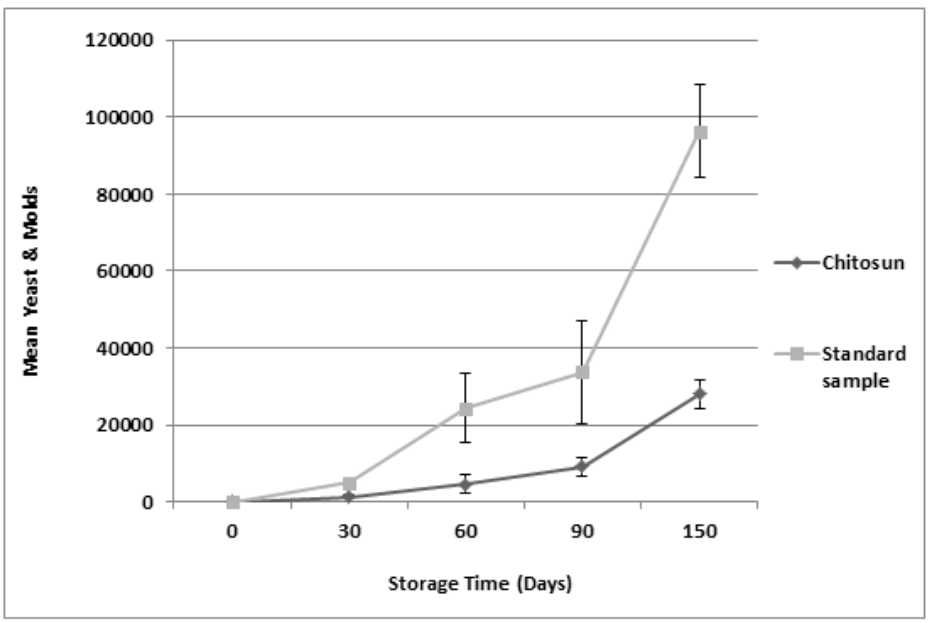


Figure 2: Changes in Yeast and Molds of caviar sample during storage time

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Total Volatile Basic Nitrogen (TVB-N)

According to Iranian national standard, maximal permissible limit is 30mg TVB-N/100gr of caviar. The TVB-N value of control samples was 28.57, 35.31 and 42mg/100gr on the 60th, 90th, and 150th days of storage time respectively, while the chitosan coated caviar TVB-N value reached to 21.8, 24.9, and 28.09 mg/100gr of caviar on the same storage time. Variation in TVB-N value are shown in Figure 5.

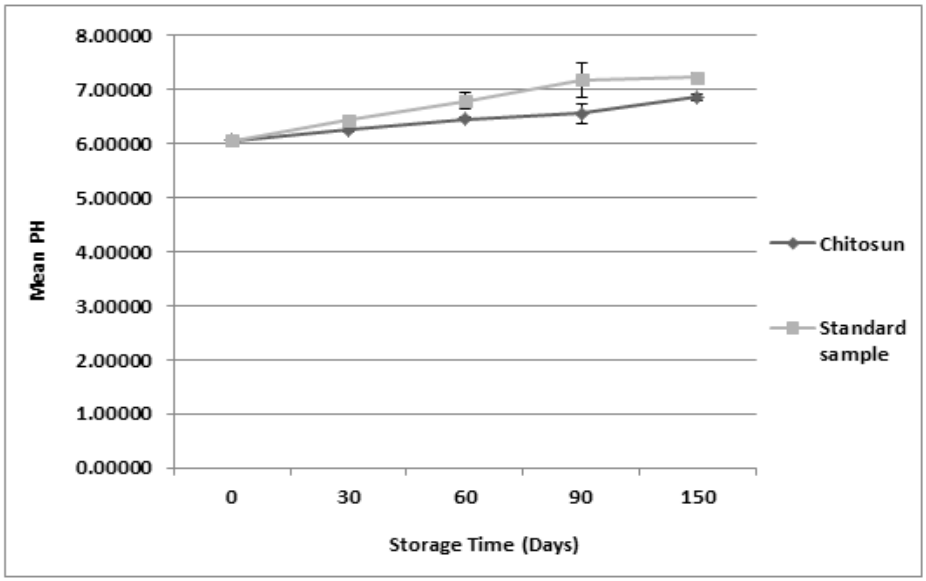


Figure 3: Changes in PH of caviar sample during storage time

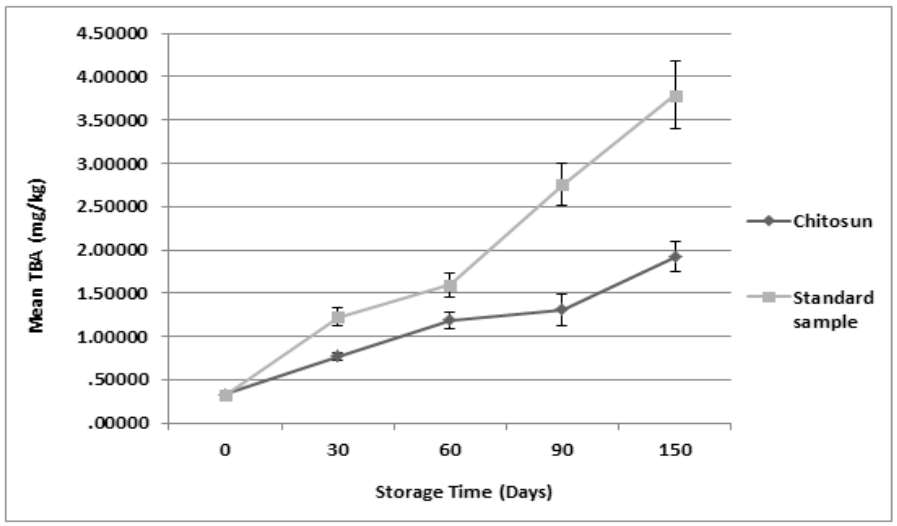


Figure 4:Changes in TBA of caviar sample during storage time

Peroxide Value (PV)

Peroxides value of control sample reached about 1.75, 2.74, 4.05, 5.34meq O2/kg on the 30th, 60th, 90th, and 150th days of storage time respectively, while the chitosan coated caviar reached to 1.09, 1.74, 2.16, 2.58meq O2/kg on the same storage time. Variation of PV value are shown in Figure 6.

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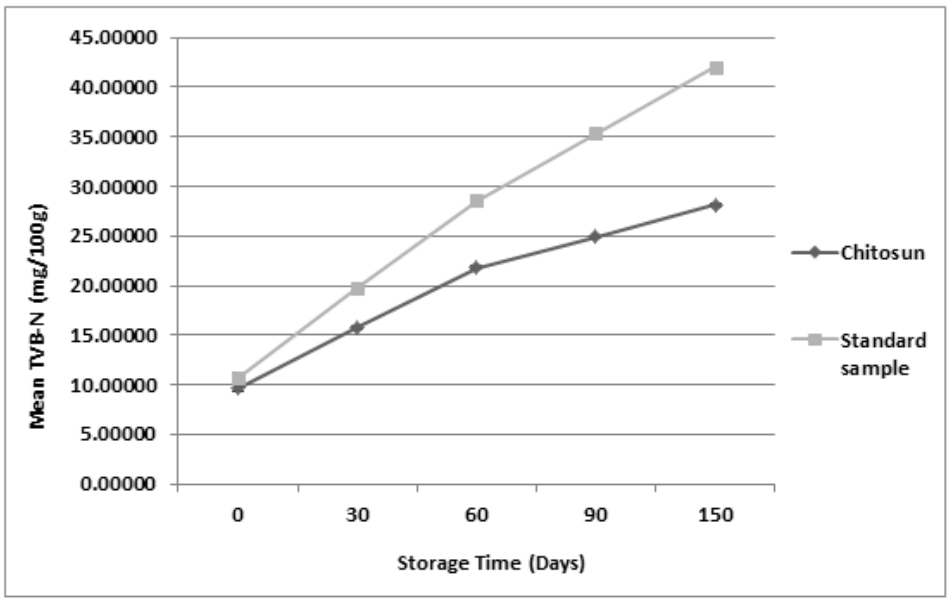


Figure 5: Changes in TVB-N of caviar sample during storage time

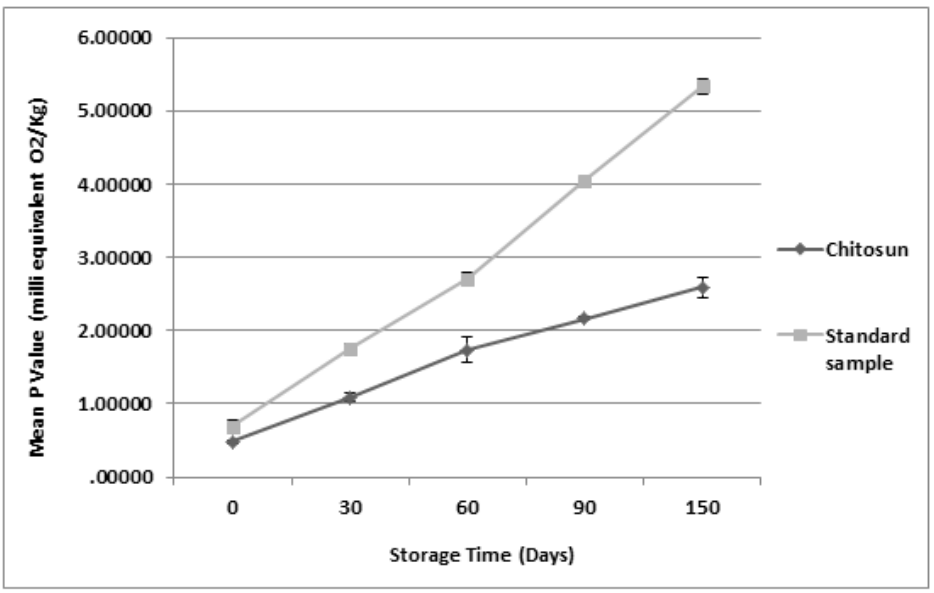


Figure 6: Changes in PV of caviar sample during storage time

Changes in Free Fatty Acid (FFA)

The FFA formation in the chilled storage condition resulted from lipid hydrolyses. Changes in FFA of caviar sample during storage time. the FFA of control samples reached about 0.89, 1.32, 2.12, 2.86 % oleic acid on the 30th, 60th, 90th, and 150th days of storage time respectively, while the chitosan coated caviar reached to 0.56, 0.76, 1.24, 1.68 % oleic acid on the same storage time (Fig. 7).

Sensory Evaluation

The sensory qualities of caviar samples were evaluated in terms of texture, flavor and general acceptability. Five-point hedonic scale (1, supper bad to 5, supper good) be used by 14 trained and professional caviar tester persons. The

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sensory score 3 and higher than 3 was defined as an acceptable caviar for human consumption. Changes in sensory score of caviar during the storage time are shown in Figure 8.

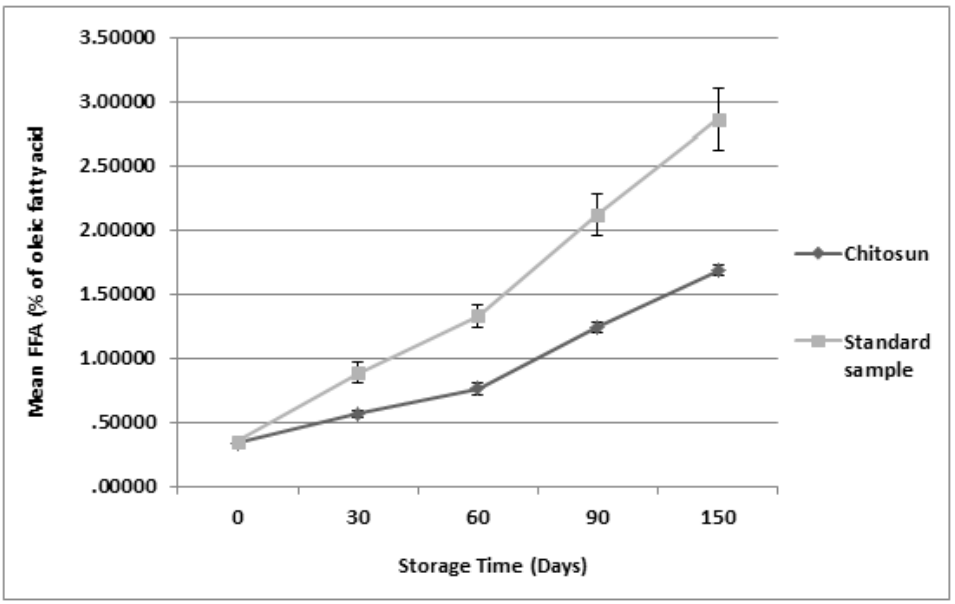


Figure 7: Changes in FFA of caviar sample during storage time

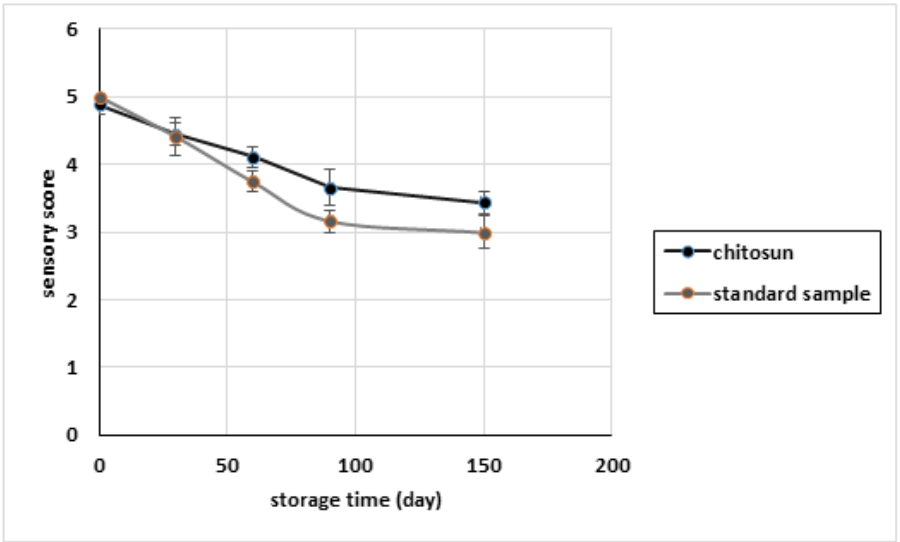


Figure 8: Changes in sensory score of caviar during the storage time

Discussion

The initial total viable count (TVC) of caviar sample was $<10^3$ CFU/g, this low number of initial TVC related to good GMP[1] and GHP[2] in caviar production. Chitosan coated sample did not exceed the maximal permissible limit of 5 log10 CFU/gr for the bacterial count on the 150th days of storage time according to Iranian National Standard and have the

[1] -Good Manufacturing Practice

[2] -Good Hygiene Practice

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better bacterial quality than the control sample on the 90th and 150th days of storage time, respectively.

As same as the bacterial count, Yeastand molds increasing in caviar coated sample was very slower than control sample and compared 4.4 log10 CFU/gr on the 150th day with control sample 4.5 log1o CFU/gr and 4.9 log10CFU/gr on the 90th and150th days of storage time, respectively.

The results of comparing data of two samples showed that coating of caviar by chitosan solution2% was effective to retention good quality factors such as TVC, Yeastand Molds at least 150 days in contrast with 90days for the control sample. The significant reduction of growing rate of TVC (p<0.01) in the chitosan coated caviar related to effective antimicrobial property of chitosan on spoilage bacteria.

The PH increasing in caviar sample was assumed to be related to increasing of volatile bases produced such as ammonia and trimethylamine by microbial or endogenous enzymes (Chaijan *et al.*, 2005). The results showed that the coated and standard sample have the same growth pattern but the important point is that rate growth of increasing PH in coated sample was very slower than standard sample during the storage time (p<0.05). It is demonstrated that PH value on the 150th day in chitosan coated was very lower than of control sample on the 90th and 150th days of storage time respectively.

Thiobarbituric acid (TBA) is an index of lipid oxidation. The rancid off-flavor became noticeable at 2mg MDA/kg threshold (Buyn *et al.*, 2003). TBA value of the control sample was significantly higher than the coated sample during the storage time (p<0.05). The initial value of TBA in control sample was 0.32mg MDA/kg and this value as other shelf life factors intend to increase during storage time and reached to1.22 and1.6mg MDA/kg after30 and 60 days of the storage time. After 90and 150 days this value reached to 2.42 and 3,78mg MDA/kg, respectively, which exceeded the maximal permissible limit of 2mg MDA/kg in the caviar. However, the final TBA value of caviar coated by chitosan was 1.31 and 1.92 mg MDA/kg after 90 and 150 days of storage time and this value was in the range of limit value in the period of storage time. These results indicated that chitosan as a natural packaging substance obviously inhibited lipid oxidation in caviar.

TVB-N is one of the important indexes to identify the quality of fresh or frozen fish because its increase is related to spoilage by bacteria and activity of endogenous enzymes (Vareltzis and *et al.*, 1997). The initial TVB-N value was 9-10mg/100gr caviar and as another spoilage factors increased during the storage time but the rate of increasing is significantly (p<0.05) different for both caviar samples. The TVB-N value of control samples was 28.57, 35.31 and 42mg/100gr on the 60th, 90th, and 150th days of storage time respectively, and exceed the upper acceptability limit after 90 days of storage time, while the TVB-N value in coated sample after 150 days reached to 28.09mg/100 caviar and was in the range of acceptability limit during

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the storage time. The results showed that chitosan coating can lead to retention good quality (TVB-N) of caviar about 60 days.

Peroxides are the primary product of lipid oxidation and play an important role in lipid auto oxidation and deteriorate them into carbonyls and other compounds (Jayakumar *et al.*, 2016). In this study PV as another spoilage factors showed a gradual increase in all of the samples. The initial PV of caviar was 0.59meq O2/kg and in control sample it reached to 4.5 and 5.34meq O2/kg on 90 and 150 days of the storage time. While maximal peroxide value of coated sample after 150 days reached to 2.59meq O2/kg during the storage time because increasing rate of PV in coated sample was very significantly (p<0.05) slower than control sample. However, data showed final PV of both samples was in the range of permissible limit (10meq O2/kg) which accepted for oils and fats (Romeo *et al.*, 2007).

Production of free fatty acids (FFAs) occurs due to hydrolysis of acylglycerols or ethyl esters during storage time. FFA increasing could be induce the textural changes, enhanced oxidation and off-favor in foods The levels of FFAs in marine lipids might reflect the degree of quality deterioration of the product, it may also affect lipid oxidation due to the fact that liberated FFA which are very sensitive to oxidation (Hamilton, 1989; Aubourg, 1993). Initial FFA was found to be (.33 % oleic acid) and it increased to 2.86 on150th and of storage time for control sample. While FFA increasing for coated sample was very slower than control sample and maximum PV on 150th day reached to 1.68 % oleic acid. The final FFA values of both caviar samples after 150 days of storage time was in the range of permissible limit value of 3-4 % oleic acid accepted for oils and fats (Toloui *et al.*, 2012). The results showed that increasing rate of FFA in chitosan coated caviar significantly (p<0.05) is slower than control sample. It is concluded that antioxidant properties of chitosan could reduce lipid decomposition in the chitosan coated caviar.

Sensory scores showed a decline in both samples with increasing storage time and the coated sample received a higher score than the control sample. It is well known that caviar spoilage gives rise to the subsequent development of strongly fishy, rancid and putrid odors, and caviar were rejected for consumption by any taste panel. The results showed control sample was acceptable up to 90 days while coated sample was in good and acceptable condition during the entire 150 days of storage time. This may be related to chitosan's functional properties such as antioxidant, antimicrobial property. Thus, the chemical quality analyses of caviar could be verified and estimated by sensory evaluation.

Chitosan is a natural and none synthetic antibacterial in recent years which has been welcomed by consumers and food industry as a result of its renewable, nonpoisonous and natural resource. However, little work has been reported on antimicrobial chitosan

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properties against microorganisms in caviar within storage time. The results of microbiological (total viable count, yeastand mold)), chemical (PH,TBA,TVN, P-value ,FFA), and sensory evaluation analyses indicated that chitosan coating on caviar could lead to maintain good quality characteristics and extension caviar shelf life at least 60 days in contrast with standard sample during the storage time.

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