

## SHELF-LIFE DETERMINATION OF BRINED GOLDEN MULLET *LIZA AURATA* DURING VACUUM REFRIGERATED STORAGE USING SOME QUALITY ASPECTS

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### ABSTRACT

**Background.** Salted fish products are popular in many countries around the world. Salting is one of the oldest techniques for fish preservation, and is essentially intended to increase the shelf-life of the product depressing water activity by means of dehydration and salt uptake by the fish muscle. However, the current demand for salted fish is driven more by the flavour of the product than for preservation purposes. Vacuum-packaging represents a static form of hypobaric storage. It is widely used in the food industry because of its effectiveness in reducing oxidative reactions in the product at relatively low cost. Low temperature storage is one of the primary methods to maintain fish quality, based on the reduction in the rates of microbiological, chemical and biochemical changes.

**Material and methods.** Fresh Golden mullets were rapidly beheaded, scaled, gutted and immediately washed with tap water then, samples were taken to the laboratory in ice box for chemical and microbial analysis of fresh fish, other samples were put in the brine (6 liter water and 2160 g salt was used for brine solution). After 14 days of brining, fish were taken out of brine solution and drained, then they were Vacuum Packed and labelled (each pack contained two fish about 1500 g weight). All the packs were stored in a refrigerator 4°C. Some quality aspects including Total Volatile Nitrogen (TVN), Peroxide Value (PV), Thiobarbituric Acid (TBA), Total Viable Count (TVC), Halophilic Bacteria (HB) and presence of *Clostridium Botulinum* were determined in fresh mullets, fresh brined mullets after 14 days of brining, and in (Vacuum Packed) VP samples stored at 4°C at intervals of 30, 60 and 90 days.

**Results.** TVN increased from ten mg/100 g in fresh brined after 14 days to 30.80 mg/100 g in VP brined Golden mullet after 90 days of storage at 4°C, PV increased after brining from 1.50 meq/kg in fresh brined to 28.90 meq/kg in VP brined Golden mullet after 90 days of storage at 4°C, TBA increased from 0.07 mg MDA/kg in fresh brined to 0.10 after 60 days and then, decreased to 0.09 mg MDA/kg in VP brined Golden mullet after 90 days of storage and TVC decreased from 4.70 log CFU/gr in fresh brined to 4.40 log CFU/gr after 30 days and then, increased to 5.70 log CFU/gr in VP brined Golden mullet after 90 days of storage at 4°C, HB increased from 4.55 log CFU/gr in fresh brined to 6.30 log CFU/gr after 90 days of storage period at 4°C and exceeded the permissible level. *Clostridium botulinum* toxin was not detected in any of the samples throughout the storage.

**Conclusions.** The results from this study clearly suggested that a combination of brining, vacuum packaging and storage at refrigerated temperature prolongs the shelf-life of Golden mullet to a great extent. Our findings revealed that the longest shelf-life was for VP brined Golden mullet stored at 4°C is 30 days.

**Key words:** brining, chemical changes, microbial growth, *Clostridium botulinum*

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## INTRODUCTION

Fish are recognised as being highly perishable, having a relatively short shelf-life [Fey and Regenstein 1982]. The spoilage of fish is a complex process in which physical, chemical and microbiological mechanisms are implicated. Enzymatic and chemical reactions are usually responsible for the initial loss of freshness, whereas microbial activity is responsible for the overt spoilage which thereby establishes product shelf-life [Gram and Huss 1996 a, b].

Salting is one of the oldest techniques for fish preservation, and is essentially intended to increase the shelf-life of the product depressing water activity by means of dehydration and salt uptake by the fish muscle, in addition, it is a preliminary operation in some smoking, drying and marinating processes [Ismail and Wootton 1992], that have been mostly empirically developed and has remained unchanged for millennia. However, the current demand for salted fish is driven more by the flavour of the product than for preservation purposes [Mujaffar and Sankat 2005]. Length of salting period as well as salt concentration depends on the expected final product [Bellagha et al. 2007]. During storage, the quality of fish degrades due to a complex process in which physical, chemical and microbiological forms of deterioration are implicated [Gonza'lez et al. 2005]. Enzymatic and chemical reactions are usually responsible for the initial loss of freshness where microbial activity is responsible for the obvious spoilage and thereby establishes product shelf-life [Gram and Huss 1996 a, b]. Vacuum-packaging represents a static form of hypobaric storage. It is widely used in the food industry because of its effectiveness in reducing oxidative reactions in the product at relatively low cost [Gopal et al. 1999]. During the two recent decades total amount of Golden mullet caught in North of Iran was about 4000 tons, that reached to 6500 tons in 2003 [Abdulmaleki and Ghaninejad 2003]. Like most of the fish in north of Iran Golden mullet is marketed fresh as whole fish, packed on ice.

The objective of this study was to investigate longest shelf-life of Golden mullet employing vacuum packaging and refrigerated storage by considering some quality aspect.

## MATERIAL AND METHODS

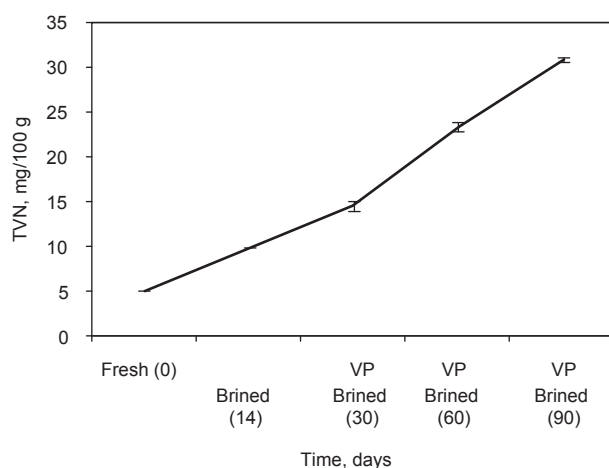
Golden mullets (*liza aurata*) were obtained from a local fisherman in Ghaemshahr City, (Mazandaran province, North of Iran). The average total length of Golden mullets were  $25 \pm 0.12$  cm and the average weight of them were about  $600 \pm 19.7$  g. Fresh fish were rapidly beheaded, scaled, gutted and immediately washed with tap water then, samples were taken to the laboratory in ice box for chemical and microbial analysis of fresh fish, other samples were put in the brine (6 liter water and 2160 g salt was used for brine solution). After 14 days of brining, fish were taken out of brine solution and drained, then they were vacuum packed using an automatic vacuum packaging machine (Mini Pack\_Italia) and labelled (each pack contained two fish about 1500 g weight). All the packs were stored in a refrigerator 4°C. At intervals of 30, 60 and 90 days VP brined Golden mullets were analysed. At each sampling time, three packs of fish were taken out and analysed. All measurements were carried out in triplicate.

TVN and PV were determined using the method described by AOAC [2000]. TBA value was determined as described by Varlik et al. [1993]. TVC and HB were determined in Plate Count Agar by the spread plate method [AOAC 2002]. Fish samples were tested for the presence of *Clostridium Botulinum* using the method described by Baker et al. [1990]. All colonies were counted and the data were reported as Colony Forming Units (CFU) per gram. Concerning microbial load, VP brined Golden mullets were analysed immediately upon opening of the packages. The values for the chemical and microbial composition of all samples were compared by analysis of variance (ANOVA). If significant differences ( $P < 0.05$ ), among means were obtained, Tukey's honest significant test was used to differentiate among means. Statistical processing was done by the SPSS computer program.

## RESULTS

The changes in TVN for all samples of VP brined Golden mullet are shown in Figure 1. Significant differences in TVN values were observed in all samples during storage ( $P < 0.05$ ). TVN increased from ten mg/100 g in fresh brined after 14 days to 30.80

mg/100 g in VP brined Golden mullet after 90 days of storage at 4°C. The changes in PV for all samples of VP brined Golden mullet are shown in Table 1. Significant differences in PV were observed in all samples



**Fig. 1.** Changes in TVN values in VP brined Golden mullet during storage (at 4°C; mean ±SD)

during storage ( $P < 0.05$ ). PV increased after brining from 1.50 meq/kg in fresh brined to 28.90 meq/kg in VP brined Golden mullet after 90 days of storage at 4°C. The changes in TBA for all samples of VP brined Golden mullet are shown in Table 1. Significant differences in TBA were observed in all samples during storage ( $P < 0.05$ ). TBA increased from 0.07 mg MDA/kg in fresh brined to 0.10 after 60 days and then, decreased to 0.09 mg MDA/kg in VP brined Golden mullet after 90 days of storage. The changes in TVC and HBC for all samples of VP brined Golden mullet during the 90 days of storage at 4°C are shown in Table 2. Increasing in TVC and HB in all samples during storage were not found to be statistically significant throughout storage ( $P > 0.05$ ). TVC decreased from 4.70 log CFU/gr in fresh brined to 4.40 log CFU/gr after 30 days and then, increased to 5.70 log CFU/gr in VP brined Golden mullet after 90 days of storage at 4°C. HB increased from 4.55 log CFU/gr in fresh brined to 6.30 log CFU/gr after 90 days of storage period at 4°C, and exceeded the permissible level. *Clostridium botulinum* toxin was not detected in any of the samples throughout the storage.

**Table 1.** Changes in PV and TBA in VP brined Golden mullet during storage (at 4°C)

Fat deterioration	Storage period, days				
	fresh (0)	brined (14)	brined vacuumed (30)	brined vacuumed (60)	brined vacuumed (90)
PV, meq/kg	0.60 ±0.00	1.50 ±0.10	3.90 ±0.00	21.02 ±0.03	28.90 ±0.11
TBA, mg MDA/kg	0.06 ±0.00	0.07 ±0.00	0.09 ±0.00	0.10 ±0.00	0.09 ±0.00

N = 3, mean ±SD. The data are expressed as the average of three samples.

**Table 2.** Changes in TVC and HB in VP brined Golden mullet during storage (at 4°C)

Microbial changes	Storage period, days				
	fresh (0)	brined (14)	brined vacuumed (30)	brined vacuumed (60)	brined vacuumed (90)
TVC, log CFU/gr	4.00 ±0.00	4.70 ±0.21	4.40 ±0.03	5.00 ±0.07	5.70 ±0.05
HB, log CFU/gr	3.30 ±0.01	4.55 ±0.02	4.75 ±0.03	5.90 ±0.03	6.30 ±0.22
<i>Clostridium botulinum</i> , log CFU/gr	0	0	0	0	0

N = 3, mean ±SD. The data are expressed as the average of three samples.

## DISCUSSION

Significant differences in TVN values were observed in all samples during storage ( $P < 0.05$ ). TVN increased from ten mg/100 g in fresh brined to 30.80 mg/100 g in VP brined Golden mullet after 90 days of storage at 4°C and reached the maximal permissible level of 30 mg/100 g [Gill 1990]. The increase in TVN was caused by a combination of microbiological and autolytic deamination of amino acids [Truelstrup et al. 1996]. The higher brine concentration would provoke denaturation of the proteins [Barat et al. 2003]. In salted fish, denaturation of proteins always occurs, but normally it proceeds more slowly than the penetration of the salt [Tülsner 1978]. The Na<sup>+</sup> and Cl<sup>-</sup> ions act as counterions toward negatively and positively charged groups, respectively, disturbing the native conformation of the proteins [Sikorski and Ruitter 1994]. The salt penetration throughout the flesh causes swelling of the myofibrillar matrix, though this is restricted in intact muscle by the sarcolemma. Denaturation by salt and by change in pH results in a decreased extractability of fish muscle proteins [Shewan 1955]. A comparable pattern of increase in TVN has been reported by Goulas and Kontominas [2005] in brined chub mackerel *Scomber japonicus* during refrigerated storage. In this study, TVN values of all samples increased as the storage time was increased and after 90 days of storage at 4°C TVN exceeded the permissible level.

Peroxide Value (PV) is one of the most important factors responsible for deterioration of fish during storage [Serdaroglu and Felekoglu 2005]. A suggested limit of PV for quality and acceptability of oils for human consumption is 8 meq/kg [Boran et al. 2006]. PV increased after brining in this study and increased from 1.50 meq/kg in fresh brined to 28.90 meq/kg in VP brined Golden mullet after 90 days of storage at 4°C. NaCl could accelerate the enzymatic oxidation [Apgar and Hultin 1982]. NaCl is able to catalyze lipid oxidation in muscle tissue [Nambudiry 1980]. Alternatively, the Na<sup>+</sup> may replace iron from a cellular complex via an ion exchange reaction [Kanner and Kinsella 1983]. The displaced iron may then participate in the initiation of lipid oxidation [Hultin 1992]. It is most likely that meat or meat products containing salt such as surimi and cured meat are susceptible to lipid oxidation [Lanier 2000]. NaCl pro-oxidant activity

accelerate the development of lipid oxidation in salted fatty fish products [Aubourg and Ugliano 2002, Goulas and Kontominas 2005]. The lipid oxidation end products themselves and their interactions with the fish muscle proteins influence the sensory properties of the product. Traces of iron, copper, calcium and magnesium ions originating in the salt, the processing equipment and/or in the tap water used in the process may accelerate both lipid oxidation and protein denaturation processes of the salted product. The use of antioxidants may forestall the lipid oxidation [Martinsen 1995, Joensen et al. 1996]. In addition, Aubourg and Ugliano [2002] reported strong effect of NaCl content on rancidity development of brined horse mackerel (*Trachurus trachurus*) during frozen storage for 270 days. In this study, PV exceeded the permissible level after 30 days of storage at 4°C.

The TBA index is a measure of malonaldehyde (MDA) content, one of the degradation products of lipid hydroperoxides, formed during the oxidation process of polyunsaturated fatty acids [Gomes et al. 2003]. Arashisar et al. [2004] proposed MDA, a secondary product of lipid oxidation, as a suitable indicator of fish meat freshness. A TBA value in the range 1-2 mg malonaldehyde/kg of fish sample is usually taken as the limit of acceptability [Lakshmanan 2000]. In this study, TBA increased from 0.07 mg MDA/kg in fresh brined to 0.10 after 60 days and, then decreased to 0.09 mg MDA/kg in VP brined Golden mullet after 90 days of storage at 4°C. TBA is not stable for long periods of time decrease in TBA values may be caused by interaction among MDA and proteins, amino acids, glycogen etc [Fernández et al. 1997]. Many factors such as species, evisceration, fat content and fatty acid composition affect TBA, which is probably the reason that acceptable limits cannot be easily set [Ruiz-Cappillas et al. 2001]. Resulting in lower amount of free MDA has been reported previously [Gomes and Moral 2003]. Storage time and atmosphere have significant effects on TBA values [Arashisar et al. 2004]. In this study, TBA values did not reach permissible level throughout the storage at 4°C.

TVC is defined as the number of bacteria (CFU/gr) in a food product obtained under optimal conditions of culturing [Huss 1993]. Value of 6 log CFU/gr was assumed to be at or near spoilage [Arashisar et al. 2004, Özogul et al. 2004]. In this study, TVC decreased from

4.70 log CFU/gr in fresh brined to 4.40 log CFU/gr after 30 days and then, increased to 5.70 log CFU/gr in VP brined Golden mullet after 90 days of storage at 4°C. Decrease in TVC after vacuum packaging was due to reduction of aerobic count of bacteria. HB increased from 4.55 log CFU/gr in fresh brined to 6.30 log CFU/gr after 90 days of storage period at 4°C and exceeded the permissible level, with respect to all the microbial variation, delayed microbial growth has been seen in all samples up to the end of storage, and these results are in agreement with other studies that observed delayed microbial growth in rainbow trout *Oncorhynchus mykiss* [Arashisar et al. 2004], swordfish *Xiphias gladius* [Pantazi et al. 2008], and sardines *Sardina pilchardus* [Özogul et al. 2004]. TVC values were found to be within the limit throughout the storage, but HB values exceeded the permissible level after 90 days of storage at 4°C.

*Clostridium botulinum* toxin was not detected in any of the samples throughout the storage at 4°C, which indicates that there was no abuse of temperature during storage. This negative result for *C. botulinum* toxin assay is in accordance with work reported by Lilly and Kautter [1990] for vacuum-packed fish fillets and modified atmospheric packed salmon fillets by Reddy et al. [1997].

## CONCLUSIONS

The results from this study clearly suggested that a combination of brining, vacuum packaging and storage at refrigerated temperature prolongs the shelf-life of Golden mullet to a great extent. Our findings revealed that the longest shelf-life for VP brined Golden mullet stored at 4°C is 30 days.

## ACKNOWLEDGEMENTS

We would like to express my sincere appreciation to Dr. Sohrab Moeini Faculty members of Islamic Azad University, Tehran North for his invaluable guidance and Eng. Mohammad Gashtasbzadeh (Fish Processing and Packaging Factory, Kian-Mahi Khazar, Mazandaran, Iran) for his contribution in this project.

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## WPŁYW OPAKOWANIA PRÓŻNIOWEGO I PRZECHOWYWANIA CHŁODNICZEGO NA TRWAŁOŚĆ TUSZEK SOLONEGO CEFALA ŻŁOTOGŁOWEGO (*LIZA AURATA*)

### STRESZCZENIE

**Wstęp.** Solone produkty rybne są popularne w wielu krajach. Solenie jest jedną z najstarszych metod utrwalania poprzez zmniejszenie aktywności wody w wyrobie. Problemem jest smakowitość tych produktów. Pakowanie próżniowe jest przykładem przechowywania żywności w warunkach obniżonego ciśnienia. W tych warunkach można istotnie spowolnić procesy utlenienia. To stosunkowo tani proces. Równocześnie pozwala na spowolnienie niekorzystnych procesów mikrobiologicznych i biochemicznych.

**Materiał i metody.** Świeże ryby były szybko odgławiane i patroszone. Tuszki po przemyciu pod bieżącą wodą umieszczano w pojemnikach z lodem. Część surowca przeznaczono do badań mikrobiologicznych i chemicznych, a resztę umieszczano w solance (6 l wody + 2160 g soli kuchennej). Po 14 dniach tuszki były wyciągane z solanki, obsuszane i pakowane próżniowo (każda paczka zawierała dwie ryby o wadze ok. 1500 g). Wszystkie próbki przechowywano w temperaturze 4°C. W trakcie badań oceniano następujące wyróżniki: ogólną zawartość lotnych związków azotowych (TVN), liczbę nadtlenu (PV), wartość TBA, ogólną liczbę bakterii (TVC), liczbę bakterii halofilnych (HB) oraz obecność *Clostridium botulinum*. Wyróżniki badano na każdym etapie oceny próbek.

**Wyniki.** Zawartość lotnych związków azotowych wzrastała od 14 mg/100 g w surowcu przechowywanym przez 14 dni w solance do 30,80 mg/100 g w próbkach zapakowanych próżniowo i przechowywanych 90 dni w temperaturze 4°C. Wartość TBA wzrosła od 0,07 mg MDA/kg do 0,10 po 60 dniach i następnie zmniejszyła się do 0,09 MDA/kg po 90 dniach przechowywania w opakowaniu próżniowym. Podobnie zmienny wpływ czasu przechowywania zaobserwowano dla pozostałych ocenianych wyróżników. W żadnej z badanych próbek nie wykryto *Clostridium botulinum*.

**Wnioski.** Wyniki badań wskazują, że połączenie solankowania, pakowania próżniowego i przechowywania w obniżonej temperaturze (4°C) znacznie przedłuża przydatność spożywczą badanych tuszek, aż do 30 dni.

**Słowa kluczowe:** ryby, *Liza aurata*, solenie, trwałość, zmiany chemiczne, *Clostridium botulinum*

Received – Przyjęto: 7.10.2011

Accepted for print – Zaakceptowano do druku: 15.11.2011

For citation – Do cytowania

Ali M., 2012. Shelf-life determination of brined Golden mullet *Liza aurata* during vacuum refrigerated storage using some quality aspects. Acta Sci. Pol., Technol. Aliment. 1(11), 37-43.