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Dikenli yılan balığı (*Mastacembelus mastacembelus*) gravlaksının 4°C'deki muhafazasında mikrobiyolojik ve besin değerleri

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Öz

Gravlaks yöntemi, henüz ülkemizde geniş ölçüde bilinmemesine karşın, bazı ülkelerde sevilerek tüketilen bir üründür. Fırat-Dicle nehirlerinde yaşayan dikenli yılan balıklarının bu yöntem ile işlendikten sonra tüketime uygun hale getirilmesi ile tüketimi sınırlı olan bu balık türlerinin ekonomiye katkıda bulunmasının yanı sıra farklı ürünlerin sunulmasına da olanak sağlayacaktır. Çalışma da; oluşturulan ürünün besin değerleri ve mikrobiyolojik kalitesi incelenmiştir. Su, protein, yağ ve kül değerleri sırası ile ortalama, % 66, 79, % 21,2, %3,18 ve % 2,07'dir. Mikrobiyolojik yönden ortalama; toplam mezofilik aerobik bakteri 3,47 log kob/g, toplam pisikrofil bakteri 2,84 log kob/g, koliform 2,04 log kob/g ve maya-füf sayısı ise 2,45 log kob/g olarak bulunmuştur. Escherichia coli hiçbir örnekte tespit edilmemiştir. Bu değerlere göre, Gravlaks yöntemiyle hazırlanan dikenli yılan balıklarının, vakum paketleme ve 4±1°C'de 35 gün süre ile mikrobiyolojik kalitesinin tüketilebilir sınır değerlerin içinde olduğu sonucuna varılmıştır.

Anahtar kelimeler: *Yılan balığı, gravlaks yöntemi, mikrobiyolojik kalite, besin değeri.*

Microbiological quality and nutritive value of 4°C stored spiny eel (*Mastacembelus mastacembelus)* gravid

Abstract

Although the fish meat prepared with Gravlax method is not yet widely known in our country, it is a well preferred food in some countries. By making the spiny eel living on the Euphrates-Tigris rivers available for consumption after processing them with this

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method will ensure both contributing this fish species with limited consumption to the economy as well as allowing different products to be served. In the study; the nutritional values and microbiological quality of the product were investigated. The average moisture, protein, lipid and ash values are respectively; 66.79%, 21.2%, 3.12% and 2.07%. When examining microbiological quality of the product; an average 3.47 log cfu/g total mesophilic aerobic bacteria, total psychrophilic bacteria 2.84 log cfu/g, Coliform and 2.04 log cfu/g and yeast-mold, 2.45 log cfu/g were determined in the product. Escherichia coli was not found in the product. According to these values, spiny eel that prepared with gravlaks method at 4±1°C and vacuum packaging of microbiological quality were determined to be within consumable limits for 35 days.

Keywords: *Spiny Eel, gravad, microbiological quality, nutritive value.*

1. Introduction

Fish meat plays an important role in the human diet with approximately18% high-quality protein, vitamins, minerals, low saturated fat and unsaturated fatty acids like omega 3-6. However, the dietary qualities are reduced when fish is processed into fishmeal and oil. Fresh fish are highly perishable products due to their biological composition. It is susceptible to spoilage and quality loss can happen very quickly after catch. Under normal refrigerated storage conditions, the shelf life of these products is limited by enzymatic and microbiological spoilage [1-5].

Gravlax (gravad lax or gravad) is a traditional Scandinavian delicacy. The word 'gravlax' comes from the Scandinavian word gräva/grave and lax/laks salmon. During the Middle Ages, gravlax was by fisherman, who salted the salmon and lightly fermented it by burying it in the sand above the high-tide line. Gravads are ready-to-eat product made mainly from salmon, greenland halibut and trout fillets cured with salt and sugar and seasoned with pepper and dill and stored at refrigerated temperatures to cure for up to two days in plastic bags. They are then packaged, sliced or whole, under vacuum or modified atmosphere. Salt in the cure acts in three ways, firstly salt restricts the growth of some bacterial species, secondly reduces water activity by osmosis, creating a denser texture and it tenderizes salmon by breaking down the structure of protein [6-8]. Although the Gravlax method is not yet widely known in some countries, it is a product favourably consumed in some countries. There is insufficient information in the literature on which phenomena have the significant effect on the stability gravlax [8-10].

Spiny eel (*Mastacembelus mastacembelus)* is native to freshwater habitats in Africa and Asia. Spiny eel in some country is not often consumed. Making eels living on the Euphrates-Tigris River systems available for consumption after processing them with this technology will ensure both contributing this fish species with limited consumption to the economy as well as allowing different products to be served [11, 12]. This study has been intended the consumption of eel rescue uniformity and expand to cover many sectors. Similarly, little is known about the effect of the gravading process on the nutritional value of the product. Therefore, the aim of present study was to determine how the process of gravlax method spiny eel (*Mastacembelus mastacembelus*) affects the nutritional value and microbiological quality of the product.

2. Materials and methods

2.1. Sample preparation

Eel (*Mastacembelus mastacembelus*) with an average weight of 518±50 g were obtained from Keban Dam Lake in Elazig located, in the eastern Anatolia region of Turkey. Eel was gutted, cleaned and washed, after it was matured in prepared mixture (salt (100 g), sugar (200 g), dill (100 g), black paper (5 g), lemon zest (5 g), black cabbage(150 g) at a rate of 350 g of mixture/kg of fillets) at +2±1°C 48 hours and then it was cut into slices [8, 13]. Samples were vacuum packed in nylon/LDPE pouches using a Henkelman packaging machine (Boxer 42, Henkelman Ind Co., Netherland). Samples were stored in a fridge (4±1°C) and analyzed at 7-day intervals to determine the shelf life. Experiments were conducted twice and in each study three replicate samples were analysed for each treatment.

2.2. Proximate analysis

The moisture content and crude ash were determined in an oven at 103°C and 550°C, respectively, until the weight became constant. The total crude protein was determined by Kjeldahl's method [14], and the fat content was analysed according to the procedure of Bligh and Dyer [15].

2.3. Microbiological analysis

A sample of fish (10 g) was diluted with 90 ml sterile 0.1% peptone water and homogenised in a Stomacher (Model 400, Seward, London, UK) at regular speed for 2 min. For microbial enumeration, 0.1 ml samples of serial dilutions (1:10, diluent, 0.1% peptone water) of fish homogenates were spread on the surface of dry media. Total viable counts were determined using Plate Count Agar (PCA, Merck) after incubation for 48 h at 30°C. Plate count agar was used for psychrotrophic bacteria and incubated at 7°C for 10 days. For *Enterobacteriaceae*, samples were inoculated with violet red bile glucose agar and incubated at 37°C for 24 h. *Escherichia coli* was determined on Chromocult TBX agar (Merck 1.16122) by incubation at 44°C for 1 day, which was performed after an incubation period at 30°C for 4 h. Yeasts and molds were enumerated potato dextrose agar (Merck 1.10130) acidified with 10% tartaric acid after incubation at 22°C for 5 days [16, 17]. Results are expressed as a logarithm of colony forming units (log cfu) per gram of sample.

2.4. Statistical analysis

All analytical determinations were conducted on days 1, 7, 14, 21, 28 and 35. Experiments were replicated twice on different conditions with different fish samples. Each sample was analyzed three times and the mean was calculated. Data were subjected to analysis of variance. According to general linear model procedures, averages of the least squares were designated through Fisher's least significant difference test and the statistical significance level was taken as 0.05 hereby ($P < 0.05$) using SAS® 6.1 (SAS Institute, Inc., Cary, NC) [18].

3. Results and discussion

Proximate analysis of eel fillet was found 74.84% water, 19.75% protein, 1.55 ash, 2.63% fat; eel gravad was found 65.79% water, 21.2% protein, 2.07% ash and 3.12 lipid (Table 1).

Table 1. The average proximate composition of fillets and gravad.

	Water (%)	**Protein (%)**	**Ash (%)**	**Lipid (%)**
Raw Spiny Eel	74.84±1.14[a]	19.75±0.35[a]	1.55±0.7[a]	2.63±0.42[a]
Gravad Spiny Eel	65.79±0.94[b]	21.2±0.24[b]	2.07±0.25[b]	3.12±0.18[b]

Values in the same column followed by different letter are significantly different ($p<0.05$).

Proximate analysis of eel fillet was found 74.84% water, 19.75% protein, 1.55 ash, 2.63% lipid; eel gravad was found 65.79% water, 21.2% protein, 2.07% ash and 3.12 lipid (Table 1). Similar the proximate composition of eel fillets were reported by Olgunoglu et al [12]. Eel gravad contained a higher proportion of protein, ash and lipid, but less water content of eel fillet. Reductions in water value is the result of osmatic imbalance due to the addition of salt and sugar in the brine. Water flows from low salt concentration to high concentration zones resulting in less water in the fish flesh [7, 9].

The microflora, total mesophilic aerobic bacteria (TMA), total psychrophile aerobic bacteria (TPC), lactic acid bacteria (LAB), *Enterobacteriaceae* (CC), *E. coli* (EC), yeast and mold count (TMYC) of the fish fillets and gravad (the subsequent storage of gravad) were determined. In the fish fillets prior to processing, the concentrations (expressed as log10 cfu/g) were as follows: TMA: 3.9± 0.6, TPC: 3.7± 1.34, CC: 2.17 ± 0.18, SA: 1.2 ± 0.6 and TMYC: 2.6 ± 0.7, *E. coli* was not detected (Table 2).

Table 2. Concentration of total mesophilic aerobic bacteria (TMA); total psychrophile (TPC); lactic acid bacteria (LAB); coliform (CC); yeast and mold (TMYC); *Escherichia coli* (EC) in the fish meats prior to processing (log_{10} CFU/g).

	TMA	**TPC**	**CC**	**TMYC**	**EC**
Fish meats	3.9±0.6	3.7±1.34	2.17±0.18	2.6 ± 0.7	**Not Detected**

Table 3 demonstrates microbiological changes in spiny eel gravad stored for 35 days at 4±1°C. In all microbiological analyses, an increase was observed with the duration of storage.

Table 3. Concentration of total mesophilic aerobic bacteria (TMA); total psychrophile (TPC); lactic acid bacteria (LAB); coliform (CC); yeast and mold (TMYC) in the eel gravad during storage at 4±1°C (log_{10} CFU/g).

	Storage Days					
	1	**7**	**14**	**21**	**28**	**35**
TMA	3.47 ± 0.7^{a}	3.96 ± 1.42^{ab}	4.44 ± 1.54^{b}	4.6 ± 0.98^{b}	4.95 ± 1.23^{bcd}	5.93 ± 1.04^{cd}
TPC	2.84 ± 1.05^{a}	2.9 ± 0.84^{a}	3.3 ± 0.64^{b}	3.84 ± 0.54^{bc}	4 ± 1.31^{cd}	5.07 ± 1.27^{d}
CC	2.04 ± 0.24^{a}	2.3 ± 1.06^{ab}	2.6 ± 0.67^{ab}	2.9 ± 0.74^{ab}	3.3 ± 1.24^{b}	3.74 ± 1.02^{bc}
TMYC	2.45 ± 0.67^{a}	2.9 ± 1.24^{ab}	3.02 ± 1.14^{ab}	3.1 ± 1.42^{ab}	3.3 ± 1.42^{ab}	3.6 ± 1.21^{b}

Values in the same line followed by different letter are significantly different ($p<0.05$).

Total viable counts in the aquatic products are the useful tool for quality evaluation of shelf-life and post-processing contamination, while psychotropic bacteria are particularly the major group of microorganisms responsible for spoilage of fresh seafood. A concentration of up to 10^6 cfu/g of TMA has been reported to be acceptable for fish products, but there is no reported acceptable limit for the concentration TPC [19, 20]. During storage, an increase in all microorganism groups were observed. The initial value of TMA was 3.47 log CFU g^{-1}. In the present study, this limit was exceeded on day 35. The initial the psychotropic bacteria (TPC) of the gravad samples was 2.84 log cfu/g (Table 3).TPC exceeded the value of 6 log cfu/g, on 35th. The values of TMA and TPC increased significantly ($p<0.05$) during storage period. Similar results for TMA have been seen in the previous study [5, 7]. *Enterobacteriaceae*, being psychrotolerant, are capable of growing at refrigeration temperatures; however, they cannot compete well with other gram-negative spoilers [17]. It has been reported that the number of coliform group bacteria limit is 9 cfu/g for food [16]. The initial concentrations of CC was 2.04 log CFU g^{-1} and increased during storage period and reached 3.74 log CFU g^{-1} on day 35. The number of coliform group bacteria did not exceed the limit value. Similar results for CC have been seen in the study [7]. *E. coli* must not be evident in any food [21] and was not detected in this study. Moulds and yeast are widely distributed in the environment and participate as the normal food flora. TMYC count in the fish meats prior to processing was 2.06 log CFU g. The initial TMYC count was significantly reduced after the gravad process. TMYC count of eel gravad increased with increasing storage time ($p<0.05$) (Table 3).

The results indicted the gravading process applicable the eel fillets and allowed to obtain new low processed fish product. The acceptable shelf life of vacuum packed grvad eel was found to be more than 35 days. During this time, the microbiological analysis of the products were acceptability limits.

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