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Sensory and Chemical Changes in Smoked Frog (*Rana esculanta*) Leg During Cold Storage (4°C±1)

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Abstract: Frog and frog leg do not have economic importance in Turkey, so they are not consumed much. They mostly are exported to other countries. Especially frog leg is consumed at Italian and French restaurants and at the holiday villages in South shores of Turkey. Turkey is one of the most important countries that export frog. As Turkey supports the hygienic criteria of EU countries, Turkey can sell to developed countries such as France, Belgium, Italy and Switzerland. In this study, it is aimed to assess whether the frog leg is proper for smoking process and to detect the nutrient composition and quality changes that occur during the 4°C±1 storage of smoked frog leg. In this study, the analyses were done with two repeats. Different parameters such as protein (%), crude lipid, crude ash, moisture (%), salt, pH, TVB-N, sensory during the cold storage were analysed. The crude protein, moisture, crude lipid, crude ash, salt, sensory score, TVB-N and pH contents of fresh frog were 22.21, 79.47, 1.05, 1.83, 0.66, 8.53, 11.73% mg/100 g and 5.26, respectively. While the initial sensory score, TVB-N and pH content of hot smoked frog leg were 8.71, 18.64 mg/100 g and 5.49, at the end of 17 days of storage, these values changed to 4.03, 28.13 mg/100 g and 5.87, respectively. According to the study results, frog leg was found to be proper for hot smoking process and it is detected that hot smoked frog legs (*R. esculanta*) conserved their sensorial characteristics for 15 days being not spoiled.

Key words: *Rana esculenta*, shelf life, cold storage, frog leg, smoking

INTRODUCTION

Frog legs are the major edible part and are considered by many to be a delicacy. The edible frog (*Rana esculenta*) is a name for the common European frog, also known as the common water frog or green frog. *Rana catesbeiana* (American bullfrog), *Rana tigrina* (Indian bullfrog), *Rana esculanta* (green frog), *Rana ridibunda* and Lexadactyla ocellatus are the main species on the market. Frog legs, are consumed in great quantities by many European countries. Turkey is one of the countries that export edible frogs to France. The wild caught frogs and the cultured frogs are mostly imported from Indonesia, China, Turkey, Thailand, India and Vietnam. Frog legs import was estimated around 8000 ton in 2001 among European Union and 2232 ton in 2004 and 2876 ton in 2005 for USA (Ozogul *et al*., 2008; Tokur *et al*., 2008). The cooked frog leg meat is soft in texture, white in colour and its flavour is described as lightly sweet and bearing a close resemblance to the white meat of a young chicken (No'Brega *et al*., 2007). Frog legs generally enter the kitchen as a frozen raw product, which will be cooked before being consumed (Andrews *et al*., 1977). Also, it contains low fat and high protein and mineral substances. It is much more durable than mollusc, snail and turtle meat but more undurable than butchery animal meat kinds. To process the frog meat by proper methods and to store it at the proper conditions, it needs to know much about the chemical composition of the meat and the quality changes that may occur during the process and storage. Freshness is the single attribute that most important, when assessing seafood quality. Microbiological, biochemical and sensory changes are associated with deterioration of meat quality during handling and storage (Gurkan, 2002; Çagiltay, 2004).

Smoking is a food-preservation technique that has been in use since ancient times. These early processed meat products were prepared for one purpose; their preservation for use at some time in the future. Preservation by smoking is believed to have been developed inadequately by the primitive tribes. At present; however, the effects of brining and smoking on colour and sensory perception are at least as important as the preservation effect due to the use of modern refrigerating systems (Rùra *et al*., 1998). The aim of smoking is besides giving it a desirable taste and odour, to provide a longer shelf life through the antibacterial and antioxidant effects of smoking (Sengor, 2004). There are

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three methods, which are used to smoke fish: the traditional method by combustion, at either low temperature (cold smoking <30°C) or high temperature (hot smoking >60°C); use of a high voltage electrostatic field which accelerates smoke deposition and use of liquid smoke, which lowers the content of polynuclear aromatic hydrocarbons (potently carcinogenic compounds) in liquid smoked fish (Goulas and Kontominas, 2005).

The aim of this study was to determine the initial quality of raw frog leg and to characterize biochemical and sensory changes of smoked frog leg (*Rana esculanta*) during cold storage at 4°C±1 in vacuum package.

MATERIALS AND METHODS

Raw material and processing: Frog legs (*Rana esculanta*) in this study were supplied from a factory that produces according to EU standards and exports all factory products to EU countries. Frogs had average 45 g±5.7 weight. Their viscera, nails and skins were removed. They were frozen at -35°C in the factory and were brought to laboratory in cold iced foam boxes by cold chain within 12 h.

Preparation of samples: First of all, frog legs thawed at 4°C±1. Crude protein, fat (%), moisture (%), crude ash, salt %, sensory, pH and TVB-N analyses were done to the frog legs. Secondly (before the smoking process), frog legs were immersed in a brine containing 100 g L^{-1}NaCl at 10°C for 10 h with a frog leg:brine ratio of 1:2 (w v^{-1}). Thirdly (smoking step), samples were taken from the pickling water and were strained (under normal circumstances, for 45 min). Then they were taken to semi controlled mechanical smoking oven. The temperature of this smoking oven is controlled automatically, while the humidity and density of wood smoke is controlled manually. Samples cooked for 3 h by increasing the oven temperature 10°C for each 30 min till 80°C (initial temperature was 21°C).

After cooking, heating was stopped and frog legs were smoked for 90 min with a mixture of hornbeam and poplar trees' shavings. Then 30 min of cooling process were applied in the oven. They were cold at chill room (4°C±1) and vacuum packaged (Komet PAXX, Plochingen, Germany) in plastic film bags in 150 g portions. Analyses were done to three kinds of samples: fresh samples, samples just before the smoking process and samples during the storage.

Analyses were carried on with 5 packets of each kind of samples for once in 2 days. All analytical determinations were done by four times at days of 0, 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19. Analyses were repeated at 3 months and 4 parallels were carried on.

Biochemical analysis: The chemical composition of smoked frog legs were determined Crude protein (Official Method No. 928.08), fat (Official Method No. 960.39), crude ash (Official Method No. 920.153), moisture contents (Official Method No. 985.14) and sodium chloride (Official Method No. 935.47) by standard methods of analysis (AOAC, 2000).

A pH meter (InoLab WTW 537, Moncheim, Germany) was used for the pH measurements (Manthey *et al*., 1988). The vapour distillation method was used to estimate Total Volatile Bases Nitrogen (TVB-N, mg N 100^{-1} g) and expressed as milligrams of TVB-N for 100 g fish muscle (Antonacopoulos and Vyncke, 1989). Samples were boiled with catalyst (MgO) and vapour components held with hydrochloric acid (0.1 N). The amount of TVB-N was calculated after the titration with sodium hydroxide (0.1 N).

Sensory analysis: Sensory analyses were done by 6 trained panellists. Smoked frog legs were assessed on the basis of appearance, odour, taste and texture characteristics using a nine point descriptive scale. Scale values were assessed as followings: 9 = like extremely, 8 = like much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly 3 = dislike moderately, 2 = dislike much, 1 = dislike extremely (Amerine *et al*., 1965).

Packing material: The frog legs were placed in high barrier plastic film bags (UPM-Kymmene, Valkeakoski, Finland). The characteristics of the plastic film bags were as follows: transmission rate (mL m^{-2}): O_2, 6.89; CO_2, 5.42; N_2, 2.48, at + 4°C; vapour permeability, 7.86 mL m^{-2} at 37.8±1°C, 90±2 % RH g m^{-2} days atm and packaged under vacuum for 20 sec at 1 bar.

Statistical analysis: Experiments were replicated twice on different occasions with different frog samples. Results are reported as mean values of eight determinations±Standard Deviation (SD). Statistical analyses were performed using SPSS 11.5 for Windows software. Data were subjected to Analysis of Variance (ANOVA). The Last Significant Difference (LSD) procedure was used to test the difference between means (significance was defined at $p<0.05$).

RESULTS AND DISCUSSION

Proximate evaluation: For fresh frog legs, for frog legs after smoking process and for frog legs after storage (17th day), analyses results were detected as followings, respectively: 22.21±1.19, 25.55±1.27, 25.59±1.51% protein; 79.47±0.59, 62.91±3.31, 65.03±2.46% moisture; 1.05±0.34,

1.23±0.17, 1.32±0.03% fat; 1.83±0.16, 3.12±0.02, 3.13±0.04% ash; 0.66±0.08, 3.36±1.04, 3.73±0.13% salt average values. The results of proximate analysis of the smoked frog are shown in Table 1.

A statistically significant ($p<0.05$) lower proximate composition was found in the non-smoked versus the smoked samples. Nevertheless, a few studies on the chemical composition of bullfrog meat, particularly fat and amino acid compositions, which may be related to its flavour are found in the literature. Bullfrog meat contains <1% fat and it is mainly composed of phospholipids (No'Brega *et al*., 2007). As USDA (2003) says, frog leg contains 81.90% water, 16.40% protein, 0.3% fat and 1.49% ash.

According to Halver (1986), for chemical composition of frog legs of two mostly used frogs, *Rana tigrina* and *Rana temporaria* the following results were obtained: moisture 75.55%, crude protein 19.88%, total lipid 2.40% and on dry basis crude protein content was 81.54%. Fatty acid composition of lipids extracted from bullfrogs (*Rana catesbeiana*) was investigated by Keum *et al*. (2002). Lipid contents of bullfrog legs and bodies were <1% and no seasonal variations observed.

Nutritional aspects of bullfrog legs and quality variation during frozen storage were examined by Xu *et al*. (1999) and bullfrog meat contained 79.8% moisture, 18.7% crude protein with all eight essential amino acids, 0.4 % crude fat, 1.1% ash. Schlesinger *et al*. (1984) were found 74.0 moisture, 20.0 protein and 0.6% lipid in frog leg meat contained. Changes in lipids of frog legs (from *Rana hexadactyla*) stored at-20°C for 150 days were studied by Sarvadeva and Srikar (1982). Total lipid content was approx 0.60 g/100 g meat throughout the storage period. Ozogul *et al*. (2008) were found 19.23% protein, 0.56% ash, 79.72% moisture and 0.68% lipid contained in *Rana esculanta* leg. In another study about frog (*Rana esculanta*), the initial crude protein, lipid, moisture, crude ash and PUFA contents of frog leg were found to be 17.82, 5.29, 66.68, 2.56 and 7.95%, respectively (Tokur *et al*., 2004).

Generally, it is said that fresh fish ash content changes between 1-2% and as the smoking process time shortens, fish dry substance amount increases (Kvale *et al*., 1998). Moisture content of mackerel fish after 60°C hot smoking process were detected as 58-67% (Kolodziejska *et al*., 2002).

Fresh catfish (*Clarias gariepinus*) protein content changed from 17.85-22.54% after hot smoking process, so did the fat content from 3.64-7.90%, moisture content from 77.89-65.15%, NaCl content from 0.45-2.23% and mineral substance content from 0.68-4.39% (Yanar, 2007).

For hot smoked *Sparus aurata*, protein content change from 20.65-25.67, salt content changed from 0.29-2.21% and moisture range changed from 69.96-57.45% after the storage at 3°C (Vasiliadou *et al*., 2005). Fresh tench fish (*Tinca tinca*) protein content changed from 12.68-18.38% after hot smoking process, so did the fat content from 1.11-1.56% and mineral substance content from 1.13-4.29% (Izci and Ertan, 2004). It is defined that the smoking method that is used has an important effect on the fat content of the product and smoked fillets contain more fat at high temperatures. It is also specified that the loss of fat after electrostatic smoking is excessive than the traditional smoking method (Espe *et al*., 2002).

Sensory evaluation: For fresh frog legs, for frog legs after smoking process and for frog legs after storage (17th day), analyses results were detected as followings respectively: 8.53±0.63, 8.71±0.27, 4.03±0.45 sensory analyses average values. The results of sensory changes of the smoked frog are shown in Table 2. In this study, the

Table 1: Proximate composition[a] of hot smoked frog legs during storage (at 4°C±1

Parameters	Storage periods (day)									
(%)	0	1	3	5	7	9	11	13	15	17
Protein	22.21±1.19^{a}	25.55±1.27^{b}	25.15±1.80^{b}	24.80±2.45^{b}	25.46±2.13^{b}	25.21±0.58^{b}	25.63±1.15^{b}	24.84±0.76^{b}	25.08±1.54^{b}	25.59±1.51^{b}
Moisture	79.47±0.59^{a}	62.91±3.31^{b}	64.89±3.09^{b}	65.12±2.43^{b}	65.16±2.57^{b}	64.94±2.41^{b}	65.36±2.05^{b}	66.43±3.06^{b}	65.75±2.15^{b}	65.03±2.46^{b}
Fat	1.05±0.34^{a}	1.23±0.17^{b}	1.37±0.47^{b}	1.24±0.17^{ab}	1.61±0.14^{c}	1.32±0.23^{b}	1.60±0.56^{c}	1.72±0.23^{c}	1.49±0.13^{bc}	1.32±0.03^{b}
Ash	1.83±0.16^{a}	3.12±0.02^{b}	3.27±0.02^{b}	3.24±0.11^{b}	3.47±0.11^{b}	3.4±0.07^{b}	3.25±0.19^{b}	3.08±0.04^{b}	3.25±0.16^{b}	3.13±0.04^{b}
Salt	0.66±0.08^{a}	3.36±1.04^{b}	3.54±0.81^{b}	3.80±0.42^{c}	3.45±0.93^{b}	3.84±0.35^{c}	3.68±0.64^{b}	3.51±0.45^{b}	3.32±0.30^{b}	3.73±0.13^{bc}

$^{a-c}$Values in the same line followed by different letter are significantly different ($p<0.05$), ^{A}Values represent the mean of eight determinations (n = 2X4)±SD

Table 2: Sensory changes[a] of hot smoked frog legs during storage (at 4°C±1)

Parameters	Storage periods (day)									
	0	1	3	5	7	9	11	13	15	17
Flavor	9.00±0.65^{a}	8.85±0.25^{a}	8.30±0.18^{a}	8.05±0.42^{a}	7.82±0.36a	7.28±0.35^{b}	7.05±0.42^{b}	6.25±0.47^{bc}	5.15±0.26^{c}	4.08±0.21^{d}
Texture	8.56±0.52^{a}	8.38±0.24^{a}	7.78±0.53^{a}	7.45±0.58^{ab}	7.30±0.18b	6.96±0.44^{b}	6.87±0.23^{b}	6.12±0.36^{b}	5.05±0.24^{c}	4.45±0.36^{c}
Appearance	8.72±0.45^{a}	8.50±0.52^{a}	8.14±0.36^{a}	7.66±0.14^{a}	7.45±0.56ab	7.25±0.51^{b}	6.65±0.32^{b}	5.48±0.53^{c}	5.04±0.46^{c}	4.15±0.09^{d}
Odour	8.70±0.35^{a}	8.64±0.48^{a}	8.24±0.28^{a}	8.08±0.34^{a}	7.64±0.46a	6.87±0.12^{b}	6.80±0.44^{b}	5.35±0.64^{c}	5.12±0.66^{c}	4.02±0.16^{d}
General taste	8.53±0.63^{a}	8.71±0.27^{a}	8.11±0.36^{a}	7.94±0.42^{a}	7.74±0.23a	7.11±0.52^{ab}	6.79±0.41^{b}	5.63±0.45^{c}	5.04±0.55^{c}	4.03±0.45^{d}

$^{a-d}$Values in the same line followed by different letter are significantly different ($p<0.05$), ^{A}Scale from 9-0 (9 excellent and 0 very bad). Rejection point≤4

shelf life of smoked frog leg was determined after 15 days at 4±1°C. The shelf life of different meat products varies considerably and depends primarily on the temperature of storage and the initial condition of raw material. The initial quality of the raw material its maximum possible shelf life and may be affected by its feeding habits, how it was bred and handled and so on. Once the aquatic products are caught, deterioration in quality is highly temperature dependant (Köse and Erdem, 2004). For a good sensorial quality of a smoked product, its water content should be high and salt content should be low. It's defined that at the 2°C storage of trout meatball becomes unable to consume on the 10th day and trout salad on the 16th day (Avci, 1996). Hot smoked herring fish shelf-life were detected as 14 days at 3°C, 7 at 7.5°C and 3 days at 15°C (Korkeala and Ristiniemi, 1998). Kolodziejska *et al.* (2002) defined that hot smoked mackerel fish could be stored at 2°C for 21 days and 7 days at 8°C without sensorial spoilage.

Changes in pH and TVB-N: For fresh frog legs, for frog legs after smoking process and for frog legs after storage (17th day), analyses results were detected as followings respectively: 5.26±0.13, 5.49±0.01, 5.87±0.25 pH; 11.73±1.70, 18.64±1.54, 28.13±3.16 mg/100 g TVB-N average values. The results of pH and TVB-N analysis of the smoked frog are shown in Fig. 1 and 2. During the storage period, the pH value increased according to the storage time but the pH value is not a criteria of spoilage. It has to be supported by other chemical and sensory analyses (Gürkan, 2002). It is defined that; for salmon fish, pH value excessed from 6.0-6.5 after smoking process (Hultmann *et al.*, 2004). For *Sparus aurata*, after hot smoking process and storage at 3°C, the pH value were 6.40 for fresh samples and 6.48 for smoked samples (Vasiliadou *et al.*, 2005) and for mackerel fish, after hot smoking at 60°C and storage at 2°C, the pH value excessed from 6.06-6.18 (Kolodziejska *et al.*, 2002). Similarly, Yanar (2007) found that the pH value of hot smoked catfish slightly changed from 6.74-6.81 after 24 days of storage at 4°C. The TVB-N value of fresh frog leg was measured as 11.73 mg/100 g. As expected, a significant increase ($p<0.05$) of TVB-N values (18.64 mg/100 g) was observed in the smoked product. An increase of TVB-N after smoking was most likely caused by an autolytic process which produces volatile amine compounds and bacterial spoilage. TVB-N is commonly used for the determination of the spoilage level during the storage period and the amount of TVB-N permitted in a product is regulated by the European Commission, if sensory evaluation gives any doubt about the freshness of fish (Tokur *et al.*, 2008). Plahar *et al.* (1999) smoked sardine and anchovy hot and detected the

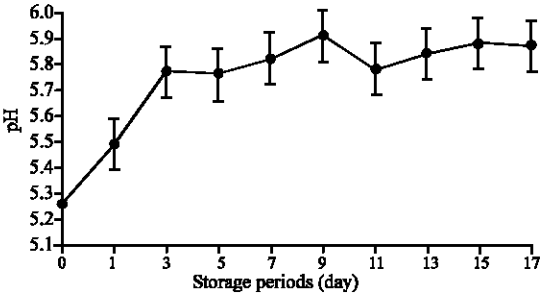

Fig. 1: Changes in the pH values[a] of hot smoked frog legs during storage (at 4°C±1)

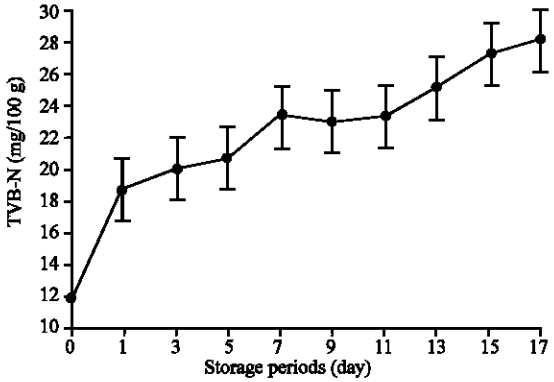

Fig. 2: Changes in the TVB-N values[a] of hot smoked frog legs during storage (at 4°C±1)

TVB-N value respectively as 27 and 32, 120 and 134 at the beginning of the smoking process and 108 and 294 mg/100 g after 6 months. In the same study, the limited TVB-N value for sea fish is given as 60 mg/100 g. Vasiliadou *et al.* (2005), determined the TVB-N value of fresh, hot smoked *Sparus aurata* samples as 18.16 and as 23.11 mg/100 g after 3°C storage. Yanar (2007) reported that the initial TVB-N value of hot smoked rainbow trout was 15.47 mg/100 g and this value increased to 29.16 mg/100 g during refrigerated storage of 24 days.

CONCLUSION

It is seen that the study is similar to other studies. The excess of pH and TVB-N values after smoking and storage are based on the forming of alkaline substances because of the protein deformation, enzyme activation and temperature. It is also clear that different results may depend on the parameters such as raw material, process conditions, smoking method, storage conditions and storage temperature. As a result of our study, it is seen that frog leg is proper to hot smoking process and preserves the limited sensorial consumability value till 17th days.

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