

Determination of Shelf Life in Fried and Boiled Frog Meat Stored in Refrigerator in $3.2 \pm 1.08^\circ\text{C}$

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Özet: Buzdolabında $3,2 \pm 1,08^\circ\text{C}$ 'da depolanan kızartılmış ve haşlanmış kurbağa etinin raf ömrünün tespiti. Bu kıyaslama çalışması farklı pişirme metodlarının bütün kurbağa eti üzerindeki etkileri üzerine yapılmıştır. Haşlama ve kızartma pişirme metodları kullanılmış örnekler raf ömrünün tespit edilmesi için buzdolabı şartlarında ($3,2 \pm 1,08^\circ\text{C}$) depolanmıştır. Haşlanmış olan örnekler kimyasal ve duyu analizler açısından kızartmış örneklerle göre daha iyi sonuçlar vermiştir. Kızartılmış ürünlerin TBA değerleri $10,60 \pm 0,12$ (mg malonaldehit/kg) bulunur iken haşlanmış örneklerde $8,16 \pm 0,57$ (mg malonaldehit/kg) olduğu görülmüştür. Depolamanın 9. gününde, duyu olarak da ransidite tespit edilmiştir. Tüm örnekler parlaklıklarını renk analiz sonucuna göre yitirmişlerdir. Doku profil analizi sonuçları göstermiştir ki sertlik, çiğnenebilirlik, elastiklik ve dış yapışkanlık parametreleri açısından kızartılmış ve haşlanmış ürünler arasında istatistiksel farklılıklar tespit edilmiştir. Kimyasal analizlere göre, kurbağa eti gerek kızartılarak gerek ise haşlanarak buzdolabında dokuz güne kadar raf ömrüne sahiptir.

Anahtar Kelimeler: Kurbağa, doku, ransidite, kızartılmış, haşlanmış.

Abstract: The comparative study was conducted on the effects of cooking methods in whole frog's meat. Boiling and frying cooking methods were used and the samples were stored in the refrigerator ($3.2 \pm 1.08^\circ\text{C}$) for determining the shelf-life. Boiling process gave the better results according to chemical and sensory analysis than fried product. TBA values of fried samples were achieved to 10.60 ± 0.12 (mg malonaldehyde/kg) when boiled samples were 8.16 ± 0.57 (mg malonaldehyde/kg). The 9th day of the storage rancidity was also determined by panelists in sensory evaluation. Both samples lost their brightness according to the color measurements. Texture profile analyses results showed us the significant differences in hardness, chewiness, springiness and adhesiveness parameters between boiled and fried samples. According to the chemical analysis, frog meat cooked by either frying or boiling were found shelf-life stable until 9th day in refrigerator storage.

Key Words: Frog, texture, rancidity, fried, boiled.

Introduction

The technological development of frog slaughtering and processing for human consumption has gained increasing attention. In Brazil the quality standards are defined by the Sanitary and Industrial Inspection Regulation of Animal Products (RIISPOA) of the Ministry of Agriculture and Land Reform (Brasil 1997), which defines frog meat within the generic denomination of 'fish' (Ramos et al., 2005). Many frog species are edible. Major frog leg suppliers include Bangladesh, Belgium, China, Indonesia, Japan, Mexico, the Netherlands and Taiwan (Andrews et al., 1977; Lutz and Avery 1999).

Normally frog meat is not only appreciated for its exquisite flavor and texture but also as a source of protein of high biological value (Vieira 1993). Although, in Brazil, frogs are marketed as whole carcass, frog meat is usually commercialized as fresh or frozen legs in the international market, with unfrozen legs obtaining higher prices (Lima et al., 1999; Pavlov et al., 1994). Furthermore, countries such as the United States, Canada and France import live animals, due to the consumers' preference for fresh meat (Lima et al., 1999). But because of the traditional habits the consumers prefer fresh meat although it is much expensive with lower shelf life.

However, detailed studies about nutritional composition of frog meat are very limited and no study available about the textural parameters and color values. Suparno et al. (1981) and Ariyani et al. (1984) reported that frog waste meal contains higher protein and fat content than fish meal. Nóbregaa et al. (2007) studied about the volatile extracts obtained from pressure-cooked bullfrog legs by simultaneous steam distillation and solvent extraction were analyzed by gas chromatography-mass spectrometry. In their results few Maillard volatiles were found, amongst them 2-acetylthiazole, the only sulfur-containing compound found in the extract. Some of these potent unsaturated aliphatic aldehydes have also been associated with chicken flavour. This may contribute to flavour resemblances between bullfrog and chicken meat. Based on estimated odour activity values, they found three most potent aroma compounds identified in the extracts were (*E,E*)-2,4-decadienal, (*E,Z*)-2,4-decadienal, and (*E,Z*)-2,6-nonadienal. Ozogul et al (2008) studied the fatty acid composition, proximate composition and mineral contents of *Rana esculata*. Due to their findings were the fatty acids occurred in the highest proportions in both the body and leg of frogs were myristic acid, palmitic acid, stearic acid, palmitoleic acid, oleic acid, linoleic acid, g-linolenic, linolenic,

cis-11,14,17-eicosatrienoic acid, cis-5,8,11,14,17-eicosapentaenoic acid and cis-4,7,10,13,16,19-docosahexaenoic acid. Among the minerals determined, potassium was found to be highest, followed by phosphorus. Calcium, aluminium, cadmium, copper, lead, chromium, nickel, boron, silicon and zinc were found to be lower than the potential toxicity levels.

In Turkey frogs are not consumed as a food. And there are five companies exporting frogs, either alive or in frozen frog legs form. Because of the large exportation amounts to Europe and America, it has an important commercial importance. The aim of the study was determining the influence of two cooking methods on the quality of frog meat during the storage period.

Materials and Methods

Frozen frogs (*Rana esculentus*) were taken from a commercial foundation and transported to the laboratory with a (-18°C) frigorific truck. It was known that they were collected from Balıkesir region in the west part of Turkey. Samples were frozen skinless, gutted and headless. 10 kg of frozen frog were taken and divided into two lots; 5 kg (170 individuals) for frying (A) and 5 kg (170 individuals) for boiling (B) process. Refrigerator thawing method was used during 18 hours for all of the samples.

For fried samples (A), frogs were covered with pasting and coating materials before frying process. Adhesive Batter + predust, (18405506) and Yellow Crumb, (18614115) patented products were used as pasting and coating materials. Samples were fried 15 minutes in a pan with sunflower oil which was 140°C. After cooling up to room temperature (25°C), samples were placed in polystyrene plates and wrapped with stretch films.

For boiled samples (B), frogs were boiled for 20 minutes in a stewpot. After reaching to the room temperature, samples were placed in polystyrene plates and wrapped with stretch films. Groups were both stored in a refrigerator (3.2±1.08°C) during the study. Temperature of the refrigerator and the inner temperature (3.6±2.18°C) of the products were measured by using data logger including thermocouple inner sensor (Testo 175-T2 Data logger).

The crude protein was determined by Kjeldahl method (AOAC, 1984). Crude fat was determined using the method of Bligh and Dyer (1959), water content and crude ash were determined in an oven at 105°C (Ludorff and Meyer 1973) and 550°C, respectively, until the weight became constant.

Total volatile basic nitrogen (TVB-N, mg N/100 g) was determined according to Vyncke (1996). Thiobarbituric acid (TBA, mg malonaldehyde/kg) was determined spectrophotometrically according to the procedure described by Tarladgis et al. (1960).

Color measurements of homogenates prepared from raw, boiled and fried (after removing batter and breading) were performed by using spectro pen (Dr. Lange) according to the Schubring (2003). For color measurements the homogenate was placed in plastic petri dishes and the color measurement was repeated ten times. In the CIE Lab system L*denotes

lightness on a 0 to 100 scale from black to white; a*, (+) red or (-) green; and b*, (+) yellow or (-) blue.

Texture Profile Analysis (TPA) was performed using TA-XT plus texture analyzer (Stable Micro Systems, Godalming, UK) according to the method of Schubring (2002). Prior to testing, samples were equilibrated to room temperature for 30 min and then they were cut into 2cm thick section. The samples were compressed twice at a crosshead speed of 0.80 mm/sec to 75% of their original height using a 5 cm diameter cylindrical probe. From the resulting force/deformation curves, the mechanical properties of hardness, cohesiveness, springiness, resilience, adhesiveness and chewiness were evaluated.

Psychotropic bacteria count (Ariyapitipun et al. 1999) and yeast-mold counts (Leroi et al. 1996) were determined in fried and boiled frog meat for microbiological analysis.

Sensory evaluation of differently processed frogs' quality was performed by five previously trained panelists by using the method of Ruiz-Capillas and Moral (2001). Sensorial attributes were appearance, flavor, juiciness, general appearance.

The SPSS (SPSS, 1999, Version 9.0. Chicago, IL, USA) program was used to determine for significant differences between mean values of the different samples. Differences between means were analyzed by one-way analysis of variance (ANOVA) followed by Tukey and Duncan tests. The results are presented as means ± SD. When a significant difference was detected between the groups (P<0.05) according to the storage, either the Tukey or Duncan multiple comparison test was applied to get the more conservative differences with multiple comparison. For comparing the groups an independent sample T- test was used to determine the differences.

Results and Discussions

Proximate compositions values of raw and processed frog groups were given in Table I. The water, crude protein, fat and ash content on a wet weight basis of raw frog meat were 78.83± 0.48 %, 18.83± 0.48 %, 1.04± 0.00 % and 0.85± 0.02 %. For comparison there is very limited literature about the nutriment content for this species. But closer studies can be seen which has done with frog waste meal. However the crude protein content of frog waste meal was found very high in the study of Tokur et al. (2008) (68.6%) and Thirumalai et al. (1971) (70.8%). This difference may be explained by the different bone and skin proportions of the waste materials. And their results were determined as dry weight basis. According to the study of Halver (1986) chemical composition values of *Rana tigrina* and *Rana temporaria* were obtained as follows; water 75.55%, crude protein 19.88% and total fat 2.40%. And also in the study of Baygar and Özgür (2010) the chemical composition of fresh *Rana esculenta* samples were determined as follows; 22.12±1.19 protein, 79.47±0.59 water, 1.05±0.34 total fat and 1.83±0.16 ash. These results were similar with the current study. On the other hand in the current study heat process of frying and boiling were used. Depending on the heat process significant differences (P<0.05) were

occurred between products in water, crude protein, fat and ash contents when comparing with raw frog and comparison between processed groups. Increased fat value on the fried group was the reason of frying process. The sun flower oil temperature was nearly 140°C and water temperature in boiling process was nearly 100°C this temperature difference in constant time caused the differences in water and fat values (Table 1).

Table 1. Proximate composition values of frogs after different cooking process

Groups	Water %	Protein%	Fat%	Ash%
Raw	78.83±0.48 ^a	18.83±0.48 ^a	1.04±0.00 ^a	0.85±0.02 ^a
Fried	52.34±0.62 ^b	34.34±0.12 ^b	8.39±0.25 ^b	1.12±0.10 ^b
Boiled	68.68±1.01 ^c	26.38±0.11 ^c	1.61±0.16 ^c	0.78±0.06 ^a

Data expressed as mean ±SD. Different superscript letters within a column are significantly different (P<0.05). N=3

TVB-N is a spoilage index for fish and seafood (FAO, 1986). FAO has indicated that samples with more than 35 mg N/100g TVB-N value are indicated as 'spoiled' (Schormuller, 1968; Ludorf and Meyer, 1973). TVB-N values in the fried and boiled frog were 20.8 and 26.6 mg N/100g, respectively on the last days of storage period. TVBN measures low-molecular-weight volatile nitrogenous compounds, such as mono-, di-, and trimethylamines and ammonia. According to the TVB-N values of both samples, no spoilage was detected during the 13 days. As a comparison in the study of Baygar and Ozgür (2010) which was about the cool storage of smoked frog, the initial TVB-N value was similar with the current study (11 mg TVB-N/100gr). And the result of their study showed us that either smoked or not frogs shelf life is not longer than 15 days in cold storage (Baygar and Ozgür, 2010). TBA index is a widely used indicator for the assessment of degree of lipid oxidation (Nishimoto et al. 1985). The thiobarbituric acid (TBA) value of frog groups were found to be 10.64 and 10.62 mg malonaldehyde/kg at the end of their storage periods. Although there is no standard on the level of TBA as an indicator of lipid quality of frog meat. Due to the study of Nishimoto et al. (1985) after 8 mg malonaldehyde/kg values are indicated in the range of spoiled. According to the TBA values on the day 9th both fried and boiled samples were spoiled (Table 2).

Table 2. TVB-N (mg N/100g) and TBA (mg malonaldehyde/kg) values of samples.

Storage Period (day)	TVB-N (mg/100g)		TBA (mg malonaldehyde/kg)	
	Fried	Boiled	Fried	Boiled
T1	10.64±0.00 ^a	9.75±0.00 ^a	1.34±0.02 ¹	1.54±0.27 ¹
T3	11.97±0.63 ^a	16.40±0.63 ^b	3.78±0.19 ¹	3.53±0.06 ²
T5	19.06±0.63 ^a	19.02±0.88 ^a	5.41±0.38 ¹	4.83±0.56 ²
T9	20.84±0.63 ^a	22.61±0.63 ^b	10.60±0.12 ¹	8.16±0.57 ²
T13	*	26.61±0.63	*	10.62±0.41

^a: Data expressed as mean ±SD. Different superscript letters within a row are significantly different for TVB-N in the same day (P<0.05). N=3. T=Day

¹: Data expressed as mean ±SD. Different superscript numbers within a row are significantly different for TBA in the same day (P<0.05). N=3. T=Day

Color values were given in Table 3. During storage period, after 3 days b* value of fried frog was significantly became higher than the boiled frog (p<0.05). For frog meat, both a* and b* values play a representative role in the evaluation of meat color, which appears to be due to the lower content of pigments in frogs' muscle. The motion of both samples were similar during the storage period. The last day of the storage period the values were found lower than the beginning day. There was an inverse correlation in a* values. In boiled samples a* value were totally climbed. On the other hand decreasing a* values can be seen in fried samples. And in both samples b* values were decreasing during the storage.

Table 3. Color values of boiled and fried samples.

Color parameters	Storage Period (day)				
	T1	T3	T5	T9	T13
Boiled L*	61.88	58.48	60.59	62.63	54.13
a*	0.12	0.51	0.20	0.39	0.30
b*	22.43	13.23	12.08	13.2	9.91
Fried L*	60.84	56.71	63.86	56.23	*
a*	3.37	2.37	1.83	1.79	*
b*	20.43	18.56	19.4	17.84	*

T=Day, n=10

The results in Table 4 showed that the hardness, chewiness, springiness and adhesiveness mechanical properties of fried group were significantly different (p<0.05) and higher than the boiled group. Cohesiveness and resilience properties of the frogs were similar (p>0.05). In a comparison between the groups fried samples were found harder and chewier.

Table 4. Texture measurements of boiled and fried frogs

Groups	Hardness (g)	Springiness	Chewiness	Cohesiveness	Resilience	Adhesiveness
Fried	325.61±52 ^a	0.30±0.05 ^a	90.37±12 ^a	0.78±0.16 ^a	0.37±0.05 ^a	-0.58±0.25 ^a
Boiled	213.24±38 ^b	0.07±0.02 ^b	17.19±09 ^b	0.91±0.18 ^a	0.43±0.03 ^a	-4.32±1.05 ^b

* ^aData expressed as mean ±SD. Different superscript letters within a column are significantly different (P<0.05). N=3

Microbiological results were given in Table V. The initial microbiological criteria of the frozen frog meat was 40 CFU/g and <10 CFU/g for psychrotrophic bacteria (PB) count and yeast-mold (YM) count, respectively. Microbial load diminished just after frying and cooking in boiling water (at day 0). At day 3, PB count of boiled frog meat increased to 3.5×10² CFU/g and it stayed at the same level until the end of day 10. At day 15, PB counts of boiled and fried frog meats reached 6.9×10³ CFU/g and 1.0×10² CFU/g, respectively whereas PB count was not detected for fried frog meat until end of day 10. YM count was not detected for both groups during the storage period. Even though the most important hazard arises from contamination with Salmonella and other fecal organisms naturally present in frogs' legs which cannot be removed entirely from the raw material before being deep-frozen

Table 5. Average bacterial counts of frog meat cooked by frying and boiling

Samples	Analysis	Storage Periods (Days)				
		T 0	T 3	T 6	T 10	T 13
Fried	PBCount(CFU/g) ^a	<10	<10	<10	<10	1.0×10 ²
	YMCCount(CFU/g) ^b	<10	<10	<10	<10	<10
Boiled	PBCount(CFU/g) ^a	<10	3.5×10 ²	2.3×10 ²	5.0×10 ²	6.9×10 ³
	YMCCount(CFU/g) ^b	<10	<10	<10	<10	<10

a: Psychotropic bacteria count, b: Yeast-mold count, T=Day, N=3

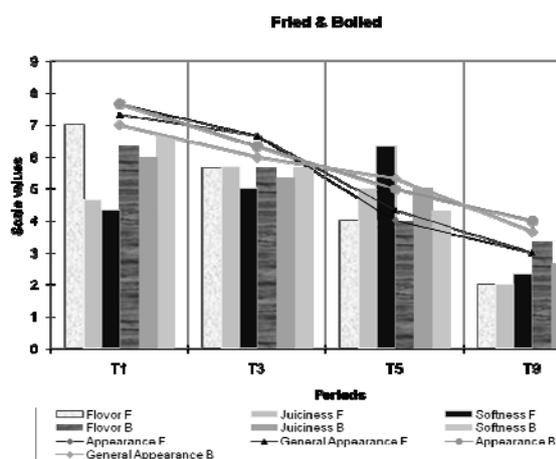


Figure 1. Sensory results

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(http://ec.europa.eu/food/fs/sc/scf/out15_en.html February 5, 2007), potentially pathogenic and spoilage flora of the deep-frozen material can be eliminated by applying adequate heat treatment. According to the microbiological analysis, frog meats cooked by either frying or boiling were found shelf-life stable until the end of 13 days storage due to the PB counts lower than 1×10⁵ CFU/g for cooked foods (ready-to-eat or with subsequent minimal heating, <70°C) (Hauschild, 1990).

Sensory evaluations of fried and boiled frogs were given in Figure 1. According to the sensory evaluation, rancidity was determined in the 9th analyze day by the panelists. These results were parallel with the determined TBA values.

They were both in good quality due to the volatile amines. According to the microbiological analysis, frog meat cooked by either frying or boiling was found quality stable after 13 days storage. But the shelf life of each lot was 9 days according to the chemical quality control analysis TBA and sensorial results.

Conclusion

Fried frog legs are very popular dish in many countries. Many luxury restaurants this meat can be found in high prices. Headless frogs can also be an opportunity for the consumers. But also determining the fatty acid composition and amino acid content of this specie may bring the literature new information in future studies.

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