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CHEMICAL COMPOSITION OF WILD AND CULTURED MARSH FROG (*RANA RIDIBUNDA*)

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Abstract

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Marsh frog has a very good meat composition and is consumed as a delicacy in countries as France, Belgium, Germany, Italy and many others. The aim of this study is to estimate chemical composition of wild and cultured water frogs. Results show that the average protein value of frog meat is relatively high, 18.52 and 22.95 g/100 g meat, respectively for wild and cultured frog. Fat content is low 0.74 and 0.93 g/100 g meat, respectively from wild and cultured frogs. Colour parameters of wild and cultured frog meat is similar (L* value = 53.83 – 50.13, a* value = 4.81 – 3.38 and b* value = 3.91 – 3.84). The content of most of amino acid is higher in meat of cultured frog then in wild frog ($P<0.05$) except non essentials amino acid serine, cysteine and alanine. The differences are reflection of higher protein content and higher sum of amino acids in meat of cultured frogs. Amino acid content of meat protein however shows higher values of leucine and proline in cultured, compare to wild frog.

It was found that meats of both wild and cultured frog had high amounts of highly unsaturated fatty acids ($C_{18:2}$, $C_{18:4,}$ $C_{20:5}$ and $C_{22:6),}$ with favourable ratio of Ω-3 to Ω-6 fatty acids. In meat of cultured frog dominated Ω-3 unsaturated fatty acid, while in wild frog meat are more Ω-6 fatty acids. Content of Important minerals like Fe, Mg, and P is higher in cultured than wild frog. Observed small differences in composition in wild and cultured frog meat reflected some difference of feeding. The results showed that cultured frogs have as valuable chemical composition as the wild frogs and other sea foods.

Key words: chemical composition, wild frog, cultured frog, *Rana ridibunda*, frog meat, amino acids, fatty acids, minerals

Introduction

Frog meat is an attractive food choice in high class restaurants. Although it has been found in European cuisine since sixteenth century, its consumption in Turkey is very low. Frogs are mainly collected from nature and with the rising human activities in twentieth century the population of the consumed frog species declined considerably. Destruction of wetland areas, uncontrolled and unsustainable capture operations and declining water resources caused by irregular rain seasons are amongst the reasons of the decline in frog populations.

The most consumed frog species with larger and bigger leg weight in Middle and Southern American and Asian countries is American bullfrog (*Rana catesbeiana*), Indian bullfrog (*Rana tigrina*), and green frog (*Rana esculanta*). These species are both collected from nature and cultured (Álvarez and Real, 2006). The Europe countries prefer a more traditionally small 50 – 60 g *Rana esculanta* and *Rana ridibunda* which are abundant in our country. Currently the

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country with the highest demand for frogs is France. France imports 3000 – 4000 tons of frozen frog legs and 700-800 tons of live frogs annually. European countries supply this demand from Turkey, Bangladesh, Albania, China, Malaysia, Indonesia and other countries (Negroni, 1997; Neveu, 2004; Neveu, 2009).

Rana ridibunda is a wild collected species in our country and it is exported as live or as frozen legs (nearly 1000 tons). There are significant number of trials and studies on culture of edible frog's species. It is essential for the cultured frog species to have similar chemical and physical properties of meat as the wild species. The aim of present study is to determined chemical composition and quality of meat of frog cultured for the first time in Turkey. Results were compared with data for the meat of nature collected wild frog.

Material and Methods

Male and female, 60-80 g *Rana ridibunda* from three main regions (Adana, Bursa and Trakya) were hand collected and brought to a private commercial frog farm. They were placed in plastic canvas covered spawning ponds (2x5.5 m). They laid eggs in May 2011. Metamorphosis was complete in 25 – 30 days. Water temperature during this period was 27±1ºC. Larvae were first fed (day 3 – day 20) with boiled spinach and lettuce. After this period, commercial trout feed was used from day 20 to day 30. Larvae which completed metamorphosis were stocked 20000 individuals/earth pond (25x5.5 m) in June 2011. Starting from this date frogs were fed with live fly larvae (*Musca domestica)* two times a day with a total 10 kg/day/pond in the morning and evening. After six months of feeding in earth ponds, frogs were harvested with a mean 45 g weight in December 2011. Mean weight of the 114 frogs used in our study was measured as 42.85 g.

Colour Analysis: Konica Minolta chromo meter (model CR 400/410; Minolta, Osaka, Japan) was used for the colour analyses. Measurements were taken from three different points on every sample. Colour measured by Hunter Lab system (L*value brightness (black to white); positive a* value red, negative green; positive b* value yellow and negative blue colour scale).

Chemical Analysis: The moisture, ash and protein values were determined according to Mattissek et al. (1992). Fat content was estimated according to modified acid hydrolysis method from Weilmeier and Regenstein (2004). Amino acid composition of the samples was analysed using high performance liquid chromatography (HPLC) according to the method described by Erkan et al. (2010). Mineral analyses in the cultured and wild frog were conducted by using Thermo X7 inductively coupled plasma mass spectrometry (ICP-MS), model X series (Winsford-Cheshire UK) according to the method described by EPA (1994). Fatty acid composition was analysed by using Thermo Trace gas chromatography (GC) according to the method described by IUPAC II D19 (IUPAC, 1980). Chemicals used in these analyses were suitable for analytical devices.

Statistical Analysis

Data analyses were carried out using the Microsoft Excel 2010 (Seattle, USA) software; one-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Test were used to compare the data according to groups at the significant level of 0.05

Results and Discussion

Colour parameters L*, a* and b* of the meat from both frog type do not differed significantly ($P>0.05$). L*, a* and b* value of wild frog meat were 53.83 ±5.30, 4.81 ± 0.80 and 3.91 ± 0.80. For the samples of cultured frogs L* value is 50.13 ±4.00, a* value 3.38 ±1.98 and b* value 3.84 ±2.60. Values reported by Cagiltay et al. (2011) for the wild collected frogs were as follows: L*value from 45.04 to 53.71; a* value from 2.99 to 3.70; b* value from 2.82 to 3.01. Therefore cultured frogs have similar properties in terms of colour characteristics as the wild frogs.

Proximate composition of the wild and cultured frogs is given in Table 1. The value of total protein was 18.52 ±0.70 and 22.95 ±0.16 g/100 g meat from wild and cultured frog respectively. Ozogul et al. (2008) reported 19.22 g protein per 100 g meat in a study conducted in Cukurova region on *Rana esculenta*. Cagiltay et al. (2011) reported 16.58 ±0.14 g/100 g, 18.94 ±0.01 g/100 g and 19.37 ±0.03 g/100 g for the wild frogs collected in Turkey from Adana, Bursa and Trakya regions respectively.

Lipid content of meat from cultured frog was 0.93 ±0.20 g/100 g vs. 0.74 ±0.15 g/100 g in wild frog meat. The difference is significant ($P<0.05$) Ozogul et al. (2008) reported fat content for *Rana esculenta* collected in Cukurova region 0.68 g/100 g. Cagiltay et al. (2011) reported in their study that *Rana ridibunda* collected in different regions of Turkey had fat content between 0.48 – 0.89 g/100 g meat.

The moisture and ash values in wild and cultured frog were found to be 79.37 ±0.50, 1.37 ±0.23 and 74.79 ±0.25, 1.0 ±0.15 respectively. These results are in agreement with data in the literature. Ozogul et al. (2008) reported 79.72 g/100 g moisture and 0.56 g/100 g ash content for wild frogs. Cagiltay et al. (2011) determined 79.04–81.67 g/100 g moisture and 0.87–1.07 g/100g ash content for wild frogs. Shearer et al. (1994) explains that the moisture, protein, fat, and ash con-

Table 1
Chemical composition of wild and cultured frogs (*Rana ridibunda*)

Proximate composition, g/100g frog meat	Wild	Cultured
Moisture	79.37 ±0.50[a]	74.79 ±0.25[b]
Protein	18.52 ±0.70[a]	22.95 ±0.16[b]
Fat	0.74 ±0.15[a]	0.93 ± 0.20[a]
Ash	1.37 ±0.23[a]	1.00 ±0.15[a]
Macro mineral, mg/100g frog meat		
Fe	0.55±0.25[a]	1.21 ±0.29[b]
Ca	24.00 ±0.25[a]	22.49 ±4.25[a]
Mg	17.00 ±0.25[a]	23.00 ±1.41[b]
P	140.00 ±6.76[a]	160.00 ±9.4[b]
Na	28.90 ±2.25[a]	48.00 ±5.43[b]
K	350.00 ±31.42[a]	366.70 ±42.51[a]
Amino acid composition, mg/100g frog meat		
Lysine	1341 ±3.18[b]	1720 ±14.18[a]
Methionine	532 ±2.23[b]	562 ±3.42[a]
Threonine	500 ±4.23[b]	744 ±5.96[a]
Isoleucine	824 ±6.18[b]	971 ±2.74[a]
Leucine	867 ±4.25[b]	1454 ±15.19[a]
Phenylalanine	795 ±3.48[a]	797 ±3.55[a]
Valine	854 ±4.63[b]	936 ±3.12[a]
Histidine	521 ±6.34[b]	573 ±8.87[a]
Serine	905 ±2.67[b]	695 ±1.28[a]
Arginine	747 ±4.75[b]	1177 ±6.07[a]
Cysteine	196 ±2.33[b]	147 ±1.03[a]
Tyrosine	615 ±2.46[b]	693 ±3.59[a]
Alanine	1302 ±1.58[b]	1011±0.35[a]
Aspartic acid	1386 ±5.78[b]	1750 ±6.47[a]
Glutamic acid	1823 ±9.04[b]	2787 ±23.01[a]
Glycine	706 ±2.08[b]	851 ±0.50[a]
Proline	373 ±2.89[b]	605 ±1.59[a]
Total amino acid	14287[b]	17473[a]
Fatty acid composition, mg/100g frog meat		
$C_{14:0}$ (myristic acid)	1.57 ±0.16[a]	1.85 ±0.35[a]
$C_{15:0}$ (pentadecanoic acid)	1.56 ±0.62[a]	1.83 ±0.39[a]
$C_{16:0}$ (palmitic acid)	125.44 ±7.84[b]	70.56 ±6.27[a]
$C_{16:1}$ (palmitoleic acid)	6.27±2.31[a]	7.84 ±0.78[a]
$C_{17:0}$ (heptadecanoic acid)	7.84±0.16[a]	7.94 ±0.11[a]
$C_{18:0}$ (stearic acid)	54.88 ±0.71[b]	74.00 ±1.33[a]
$C_{18:1}$ (oleic acid)	54.17 ±0.78[b]	75.07 ±1.56[a]
$C_{18:2}$ n-6 (linolelaidic acid)	2.35 ±0.17[a]	1.90 ±0.19[a]
$C_{18:2}$ n-6 (linoleic acid)	81.54 ±0.81[b]	38.05 ±0.23[a]
$C_{18:3}$ n-3 (linolenic acid)	15.68 ±0.11[b]	18.50 ±0.38[a]
$C_{20:1}$ (eicosanoic acid)	1.61 ±0.12[a]	1.88 ±0.19[a]
$C_{20:2}$ (cis 11,14 eicosadienoic acid)	5.49 ±0.14[a]	5.55 ±0.21[a]
$C_{20:3}$ n-3 (cis 11,14,17 eicosatrienoic acid)	1.48 ±0.09[a]	1.84 ±0.19[a]
$C_{20:4}$ n-6 (arachidonic acid)	70.56 ±0.16[b]	92.50 ±0.43[a]
$C_{20:5}$ n-3 (cis 5,8,11,14,17 eicosapentaenoic acid)	31.36 ±0.24[b]	37.00 ±0.37[a]
$C_{22:0}$ (behenic acid)	5.49 ±0.17[b]	7.40 ±0.18[a]
$C_{22:6}$ n-3 (cis 4,7,10,13,16,19 docosahexaenoic acid)	36.85 ±0.63[a]	81.40 ±0.95[a]
$C_{23:0}$ (tricosanoic acid)	3.14 ±0.08[b]	3.72 ±0.19[a]
$C_{24:1}$ (nervonic acid)	3.92 ±0.10[b]	5.55 ±0.22[a]
not identified	78.40[b]	145.00[a]
Total n-3	85.37[b]	138.74[a]
Total n-6	154.45[b]	92.50[a]

***Different letters in the same line for each groups indicate significant differences ($P<0.05$)*

tent of aquatic animals tend to change with species, climate, seasons, sexual maturity, levels and regime of feeding. This study showed the nutritional composition of cultured frogs is similar with wild specimens.

The macro element composition of wild and cultured frog meat are depicted in Table 1. Results showed that the farmed frog meat contained higher levels of macro elements magnesium, phosphorus and iron, than meat of wild frog. Ozogul et al. (2008) reported lower macro mineral content in mg per 100 g meat of wild *Rana esculenta*, 7.7 Ca, 8.97 Mg, 1.22 Na, 46.35 P and 62.1 K. There are significant variations in data for minerals content of frog meat of different authors. According to USDA National Nutrient Database (2009) frog meat has in mg per 100 g 1.5 Fe, 1.80 Ca, 20.0 Mg, 147 P, 58 Na and 285 K. Cagiltay et al. (2011) reported in mg per 100 g 0.4 – 0.6 Fe, 21 – 30 Ca, 15.53 -18 Mg, 133 -153 P, 23 – 35 Na and 236 – 267 K for collected wild *Rana ridibunda*. Our data are in ranges published by other authors. These results show that cultured frogs have similar mineral compositions as their wild counterparts. Estimates of mineral requirements for adults have been reported in milligrams per day as follows: phosphor 800, potassium 2000, sodium 500, magnesium 280, calcium 800 and iron 10 (Oksuz, 2012). Consumption of one hundred grams of wild and cultured frog portion may provide between 5% and 45% of daily mineral requirement of a human body.

Level of all amino acids, except phenylalanine, in meat of cultured frog is higher, compare to the wild frog's meat. Differences however are connected with different crude protein content. When content of amino acid is calculated as a percentage of crude protein in the meat, for most of amino acids there is not significant differences ($P > 0.05$). Cultured frog meat contain significantly more, compare to wild frog, only leucine and praline ($P<0.05$). The wild frog meat is richer then cultured one of phenylalanine and non essential amino acids serine, alanine and cysteine ($P<0.05$). Ruiz-Capillas and Moral (2004) defines that alanine, glutamic acid and glycine is responsible for the aroma and flavor.

Results of these study show that glutamic acid, aspartic acid,alanine and lysine are dominating amino acids in frog meat. This is in agreement with publications of Cagiltay et al. (2011) and Tokur et al. (2008). It is evident from this study that wild frog meat amino acid composition is almost similar to the cultured frog meat. Comparing with amino acid requirements for adults people, reported by WHO (2007), it is evident that 100 g of cultured frog meat consumption may provide more than daily amino acids requirement for adult.

The analysis of fatty acid composition of meat from frog's legs shows that palmitic acid with 70 to 125 mg/100 g and stearic acid with 55 to 74 mg/100 g are dominant saturated fatty acid. The main polyunsaturated fatty acids were oleic, linoleic, arachidonic, eicosapentaenoic and docosahexaenoic fatty acids in the meat of wild and cultured frog legs. Similar fatty acid content has been reported for frog legs by Sarvadeva and Srika (1982). In meat of cultured frog dominated Ω-3 unsaturated fatty acid, while in wild frog meat are more Ω-6 fatty acids. Probably differences are connected with quality of feed for two ways of growing frogs. Alfa-linolenic acid ($C_{18:3}$), eicosapentaenoic acid ($C_{20:5}$); docosahexaenoic acid ($C_{22:6}$) omega-3 fatty acids have an important role in human nutrition and nutritional physiology (Davis and Kris-Etherton, 2003). This fatty acids present in significant quantity in frog meat as is shown in Table 1. Cholesterol and triglycerides are lowered by omega-3 fatty acids as well as the risk for heart disease. With the help of omega-3 fatty acids, immune system and brain cells are strengthened, blood sugar is balanced, blood flow is easier and coagulation is prevented (Simopoulos, 1991; Covington, 2004). Some studies showed that brain and nervous system works more efficient with omega-3 fatty acids (Crawford, 1993). One of the most important sources for omega-3 fatty acids is salmon followed by mackerel, sardines, anchovy and tuna species (Belitz et al., 2001; Ward and Snigh, 2005). It is evident (Table 1) that cultured frog meats are rich in these types of fatty acids. Similar results are reported by Ozogul et al. (2008) on wild collected *Rana esculanta* and Cagiltay et al. (2011) on wild collected *Rana ridibunda*. The intake of total omega-3 fatty acids in the United States is ~ 1.6 g/d per person. Of this, α-linolenic acid accounts for ~1.4 g/d, and only 0.1 to 0.2 g/d comes from EPA and DHA (Kris-Etherton et al., 2002). The omega-3 content of the wild and culture frog meat (85.37 and 138.74 g/100 g) is close to perfection in this respect.

Conclusion

Results of this study of chemical composition and colour characteristic of wild and cultured *Rana ridibunda* showed that legs meat of both type of frogs' do not differ significantly in most of studied parameters. Meat of wild and cultured frog has relatively low lipid content, good amino acid, fatty acids and mineral composition as other seafood. Differences between meat of wild and culture frog are not big. Therefore, cultured frog meat has the potential to attract the attention of food technologists and dieticians as a new animal healthy food source based on its good chemical composition. Further study with frog culture merit attention will continue.

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