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Seasonal changes in proximate composition of anchovy (*Engraulis encrasicolus, L.*) from the central Adriatic

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The chemical composition of fish varies greatly depending on the starvation and intensive food intake periods and other external factors such as sea temperature. The seasonal changes define the application of different technological processes and are an aspect of quality of raw material, sensory attributes and storage stability. The results of monthly proximate composition analysis of the edible portion of anchovy were investigated over a period of two years. The average proximate composition of anchovy caught in the Adriatic Sea was as follows: water content 76.52 ± 1.38 %, protein content 21.34 ± 0.29 %, fat content 2.27 ± 1.20 %, ash content 1.42 ± 0.08 %. Protein and ash content showed small annual variations and differences within months were mostly insignificant. The fat and water content showed significant seasonal changes and a very strong negative correlation between fat and water content was observed (r = - 0.947). The fat content varied from 0.86 to 4.47 %, with a minimum in February and maximum in October. There was a statistically significant relationship between fat and water content at the 99% confidence level, indicating that this relation could be used to estimate fat content based on the analytical determination of water content.

Key words: *Engraulis encrasicolus,* proximate composition, seasonal changes, fat content

INTRODUCTION

Anchovy (*Engraulis encrasicolus*, L.) is the only European representative of the Engraulidae family and it is an endemic species of the Mediterranean-Atlantic region. It belongs to the group of small pelagic fish, caught along the Mediterranean shores and widely spread in the Adriatic Sea. The migration of anchovies from coastal regions towards open sea waters is related to their age and size, thus bigger (older) anchovies mainly inhabit the open sea. Mesenteric fat content changes during the year depending on the sexual cycle, showing the maximum values during winter months. The anchovies are caught all the year around, but mainly within the spring-autumn period (SINOVČIĆ, 2000). It represents a species of important economic value in all parts of the Mediterranean. The average capture production of European anchovy from 2002 to 2008 in the Mediterranean and Black Seas was around 480000 tonnes per year (FAO, 2010). The Croatian catch of anchovies in the Adriatic Sea increased four times since 2003 and had reached 13758 tons in 2010 (CBS, 2011). Most of the catch is processed by salting, marinating or freezing, and utilized for human consumption. The proximate composition of pelagic fish species varies with season, is affected by food intake, sexual maturity and sea temperature (GÖKOĞLU *et al*., 1999). Different authors reported high variations in fat content of the edible part of anchovy, with ranges from 0.94 % to 33.30 % wet weight (FAO, 1989; KARAÇAM & BORAN, 1996; GÖKOĞLU *et al*., 1999; KAYA & TURAN, 2010). A substantial normal variation is observed for the constituents of fish muscle. The principal constituents most affected by the seasonal cycle changes are fat and moisture. The knowledge of proximate composition of fish species is important in the application of different technological processes (HUSS, 1988) and as an aspect of quality of raw material, sensory attributes and storage stability (SIKORSKI *et al*., 1990). Randomly performed analysis of fat content in anchovies caught in the Adriatic Sea indicated that the fat content variations were not as great as those described in the literature. Therefore, the aim of this paper was to investigate the seasonal changes in proximate composition of the fillet of European anchovy caught in the Adriatic Sea, with special emphasis on fat and water content.

MATERIAL AND METHODS

Approximately 5 kg of anchovies were obtained from a local fish factory each month from January to December of 2009 and 2010. The fish were caught between 11th and 19th of each month in the central Adriatic fishing region GSA 17 (GMU 37.2.1). At every sampling the fish were placed in self-draining polystyrene boxes, packed in flake ice and delivered to the laboratory on the same day. Fifty individual samples were randomly selected from 5 kg batches, resulting in an average length of 15.23 ± 0.68 cm and an average weight of 24.72 ± 3.64 g. Fish were gutted, filleted and analysed on the same day.

The fillets were homogenised using a laboratory blender (KINEMATICA Mikrotron MB 550, Switzerland) and the homogenates were used as the representative samples for the analyses. The proximate composition was determined as water content (drying the samples to constant weight at 105°C) (POLIGNE & COLLIGAN, 2000), crude protein (Kjeldhal method, N × 6.25), crude fat (acid hydrolysis method) and crude ash (calcination at temperatures ≤ 500°C) (AOAC, 2000). All analyses were done in triplicate and presented as percentage on wet weight of anchovy fillet.

Graphic figures and statistical evaluations (analysis of variance, least significance difference, correlations) of obtained data were performed using Microsoft Office Excel 2007 package and software Statgraphics® Plus v. 5.1 Professional (Manugistics, Inc., Rockville, MD, USA).

RESULTS AND DISCUSSION

A great variation in proximate composition of some marine species from the Mediterranean has been reported (KARAKOLTSIDIS *et* al., 1995; GÖKOĞLU *et al*., 1999), yet information available on the chemical composition of marine species harvested from the Adriatic Sea is very limited however. The results of monthly proximate compositions analysis of the edible portion of anchovy were investigated over a period of two years. An average of 56.50 % of the fish consisted of edible portions. Tables 1 through 4 show water, protein, fat and ash content of anchovy fillet analysed monthly during two years. Analysis of variance (ANOVA) showed no statistical difference ($p>0.05$) between the two years. The average proximate composition of anchovy caught in the Adriatic Sea was as follows: water content 76.52 ± 1.38 %, protein content 21.34 ± 0.29 %, fat content 2.27 ± 1.20 % and ash content 1.42 ± 0.08 %. Protein and ash content showed small annual variation and differences within months were mostly insignificant ($p>0.05$). These values are in agreement with chemical composition data for anchovy (*Engraulis encrasicolus*) from Italian (CUISA & GIACCO, 1969), Spanish (FRAGA, 1966) and Turkish waters (OLGUNOĞLU *et al*., 2009), with the exception of fat content.

The chemical composition of fish varies greatly from one species and one individual to another depending on the starvation and intensive food intake periods (HUSS, 1995) and external factors such as temperature and salinity (ZLATANOS & LASKARIDIS, 2007). Variations in percentage of fat should be reflected in the percentage of water, and the two normally constitute around 80% of the fillet (HUSS, 1995).

Table 1. Changes in water content of anchovy analyzed monthly over a two year

Month	Moisture (%)					
	2009		2010		Mean	
1	77.70	(0.10)*	77.24	(0.24)	77.47	(0.17)^{A}
2	77.95	(0.35)	78.05	(0.24)	78.00	(0.30)^{B}
3	77.96	(0.25)	78.06	(0.26)	78.01	(0.25)^{B}
4	77.85	(0.25)	77.95	(0.14)	77.90	(0.20)^{B}
5	77.46	(0.03)	77.56	(0.13)	77.51	(0.13)^{C}
6	76.23	(0.23)	76.33	(0.33)	76.28	(0.28)^{D}
7	76.26	(0.64)	76.36	(0.24)	76.31	(0.24)^{D}
8	75.36	(0.64)	75.46	(0.14)	75.41	(0.22)^{E}
9	74.85	(0.03)	74.95	(0.13)	74.90	(0.23)^{F}
10	74.75	(0.07)	74.85	(0.17)	74.80	(0.17)^{F}
11	74.98	(0.14)	75.08	(0.24)	75.03	(0.19)^{F}
12	76.10	(0.44)	75.74	(0.18)	75.69	(0.19)^{G}

**Mean (standard deviation); n = 3.*
$^{A-G}$ Values in the same column and labelled with the same uppercase letter do not differ significantly

Table 2. Changes in protein content of anchovy analyzed monthly over a two year period

Month	Protein (%)					
	2009		2010		Mean	
1	20.78	(0.43)*	20.71	(0.36)	20.75	(0.39)^{A}
2	21.64	(0.22)	20.89	(0.28)	21.27	(0.25)^{AB}
3	21.19	(0.34)	21.12	(0.27)	21.15	(0.31)^{AB}
4	21.45	(0.32)	21.38	(0.25)	21.41	(0.29)^{AB}
5	20.89	(0.76)	21.45	(0.42)	21.17	(0.59)^{AB}
6	21.59	(0.39)	21.52	(0.32)	21.55	(0.35)^{AB}
7	22.15	(0.30)	21.14	(0.14)	21.64	(0.22)^{B}
8	21.28	(0.45)	21.21	(0.38)	21.24	(0.41)^{AB}
9	20.68	(0.56)	22.01	(0.40)	21.34	(0.48)^{AB}
10	21.85	(0.18)	22.06	(0.26)	21.95	(0.22)^{B}
11	21.24	(0.56)	21.31	(0.63)	21.27	(0.59)^{AB}
12	21.39	(0.42)	21.32	(0.35)	21.35	(0.38)^{AB}

**Mean (standard deviation); n = 3.*
$^{A-B}$ Values in the same column and labelled with the same uppercase letter do not differ significantly

Table 3. Changes in fat content of anchovy analyzed monthly over a two year period

Month	Fat (%)					
	2009		2010		Mean	
1	1.75	(0.07)*	1.96	(0.12)	1.86	(0.10)^{A}
2	0.80	(0.04)	1.09	(0.14)	0.95	(0.11)^{B}
3	0.93	(0.09)	1.24	(0.09)	1.09	(0.11)^{BC}
4	1.14	(0.16)	1.84	(0.12)	1.49	(0.16)^{AB}
5	1.45	(0.07)	1.96	(0.12)	1.71	(0.17)^{AC}
6	1.51	(0.11)	2.14	(0.14)	2.33	(0.14)^{AD}
7	2.57	(0.07)	2.24	(0.17)	2.41	(0.24)^{AD}
8	2.76	(0.23)	3.12	(0.20)	2.94	(0.23)^{DE}
9	3.36	(0.22)	4.09	(0.21)	3.73	(0.22)^{F}
10	4.27	(0.21)	4.24	(0.18)	4.26	(0.31)^{F}
11	2.32	(0.11)	3.84	(0.16)	3.58	(0.21)^{E}
12	1.73	(0.10)	3.01	(0.18)	2.87	(0.10)^{D}

**Mean (standard deviation); n = 3.*

$^{A-F}$ Values in the same column and labelled with the same uppercase letter do not differ significantly

Table 4. Changes in ash content of anchovy analyzed monthly over a two year period

Month	Ash (%)					
	2009		2010		Mean	
1	1.49	(0.03)*	1.41	(0.13)	1.45	(0.08)^{AB}
2	1.43	(0.04)	1.43	(0.06)	1.43	(0.05)^{ABC}
3	1.49	(0.03)	1.44	(0.09)	1.49	(0.06)^{B}
4	1.41	(0.05)	1.49	(0.04)	1.45	(0.04)^{AB}
5	1.41	(0.05)	1.34	(0.03)	1.37	(0.04)^{C}
6	1.36	(0.06)	1.36	(0.11)	1.36	(0.08)^{C}
7	1.41	(0.12)	1.39	(0.08)	1.40	(0.05)^{AC}
8	1.40	(0.04)	1.42	(0.06)	1.41	(0.06)^{AC}
9	1.38	(0.08)	1.44	(0.09)	1.41	(0.08)^{AC}
10	1.38	(0.04)	1.42	(0.11)	1.40	(0.08)^{AC}
11	1.40	(0.08)	1.39	(0.09)	1.40	(0.09)^{AC}
12	1.49	(0.09)	1.44	(0.12)	1.47	(0.07)^{AB}

**Mean (standard deviation); n = 3.*

$^{A-C}$ Values in the same column and labelled with the same uppercase letter do not differ significantly

In this research fat and water content showed significant seasonal changes (Tables 1 and 3) and constituted 78.87 ± 0.34 % of the fillet. A very strong negative correlation between fat and water content was observed ($r = -0.947$). The fat content varied from 0.86 to 4.47 %, with a minimum noted in January and a maximum in October. Significant differences between months were determined by least significant difference at the 95.00 % confidence level. A significant decrease ($p<0.05$) in fat content was recorded during the winter months, from November to April, and a significant increase from July to October. The obtained results of fat content analysis coincide with the mesenteric fat content variation of anchovy from the Adriatic Sea, with the highest values recorded in the winter months (SINOVČIĆ, 2000). The author found a direct dependence of mesenteric fat content with spawning activity of anchovy, but not to the active feed intake period. Summer months are the period of intense sexual activity of anchovy, so it is expected that the fat content is lower. Anchovy samples taken for this research were caught in open sea waters where they reach the spawning maximum sooner than samples from coastal regions. This explains the lower fat content observed in the late spring and early summer period (Table 3). Because fat content can vary widely during the spawning period, thus water content also varies. Mean water content of the anchovy fillet had its maximum in February and March, and showing an inverse relationship with fat content (Fig. 1).

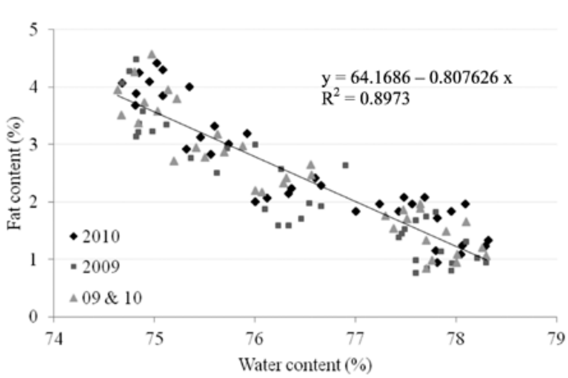

Fig. 1. Seasonal changes in fat and water content of anchovy fillet. Each point represents the mean of six values with vertical bars denoting standard deviation

Some authors reported higher fat content and wider seasonal variation in anchovy (*Engraulis encrasicolus*) caught in the Sea of Marmara of Turkey. In their study fat content varied from 5.30 to 13.60 % during the fishing season, and showing the maximum in November. The authors found a similar inverse relationship between fat and water content (GÖKOĞLU *et al.*, 1999).

The analysis and seasonal changes in proximate composition of anchovy fillet from the Adriatic Sea has shown that, regardless of variations, the fat content remained <5% all year around. When using the 5 % of total lipid level as a cut-off point between low and medium fat fish (ASHTON, 2002), Adriatic anchovy would represent low fat fish. However, these data refer to fillet composition, and anchovies store mesenteric fat in the visceral cavity, which is characteristic for fatty fish species. This is relevant in the application of different technological processes and storage because actual fat content has consequences for the technological characteristics *post-mortem* and can be used as an aspect of quality of raw material (HUSS, 1995). Fat content affects the flavour and sensory characteristics of seafood products in general. So, the relatively low fat content (<5%) of the fillet makes anchovy a good choice of raw material, thus not as submissive to lipid deterioration as other small pelagic fish with more pronounced seasonal variations.

Fat content has shown inverse proportionality to water content in some medium-fatty and fatty fish species muscle (LOVE, 1997). This would allow a rapid and indirect measurement of lipid content through water content analysis, thus helping maximize the profitability of fish (KENT, 1990). The relationship between fat and water content in anchovy fillet was investigated, taking into account the fat and water content during two years.

The results of fitting a linear model to describe the relationship between fat and water content data for two years of analysis are shown in Fig. 2. Since the P-value in the ANOVA table is less than 0.01, there was a statistically significant relationship between fat and water content

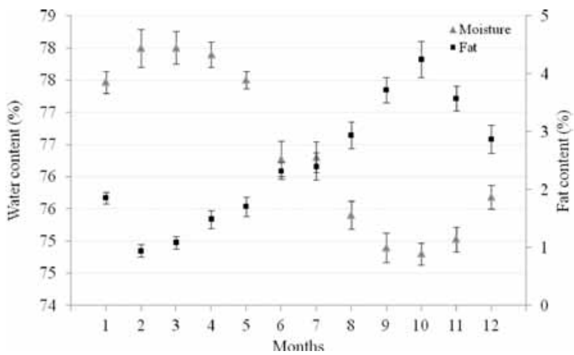

Fig. 2. Functional relationship between fat and water content in anchovy fillet period

at the 99% confidence level. The R-Squared statistic indicates that the model as fitted explains 89.73% of the variability in fat content. Given this relationship between fat and water content a simple regression equation developed based on the trend line in Fig. 2 would allow the fat content to be estimated based on the moisture content. This would not replace the standard procedures for precise measurement of fat content; however, it would allow processors to make an easy estimation of fat content. A linear relation between water and lipid content was determined for other marine fish species (KENT, 1990; LOVE, 1997; WHEELER & MORRISSEY, 2003; YESANNES & ALMANDOS, 2003).

CONCLUSIONS

This research gives important data on seasonal changes in proximate composition of the fillet of European anchovy caught in the Adriatic Sea. Since most of the catch is processed by salting, marinating or freezing and utilized for human consumption, and taking into account anchovy's important economic value, the information on monthly changes in the proximate composition during a two year period should be useful to both seafood processors and scientists in providing them better knowledge of this species and standard proximate composition. Although this research took into consideration a great number of samples over two years, reported data might be considered standard for fish size in the group of 40-42 individuals per kilogram. The proximate composition of smaller/bigger anchovy samples might be influenced by the biological differences. High protein content and relatively low fat content (<5%) of the fillet make anchovy a good choice of raw material and gives it an advantage over other small pelagic fish in which seasonal differences might be much more pronounced. Additionally, the analysis of the water content of anchovy fillet has shown to be useful in the estimation of fat content in the edible part of anchovy.

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Sezonske promjene u kemijskom sastavu brgljuna (*Engraulis encrasicolus*, L.) iz srednjeg Jadrana

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SAŽETAK

Odstupanja u kemijskom sastavu ribe značajno ovise o razdobljima gladovanja i intenzivne prehrane riba, te o ekološkim čimbenicima. Sezonske promjene određuju mogućnosti primjene različitih tehnoloških procesa i koriste se kao pokazatelj kvalitete sirovine, senzorskih svojstva i stabilnosti tijekom pohrane. U radu su istraživane razlike mjesečnih analiza kemijskog sastava jestivog dijela brgljuna kroz razdoblje od dvije godine. Dobiveni prosječni kemijski sastav brgljuna ulovljenog u Jadranskom moru je sljedeći: 76,52 ± 1,38 % vode, 21,34 ± 0,29 % bjelančevina, 2,27 ± 1,20 % masti i 1,42 ± 0,08 % pepela. Udjeli bjelančevina i pepela pokazali su malu godišnju varijabilnost, a razlike između mjeseci uglavnom nisu statistički značajne. Udjeli masti i vode pokazali su značajne sezonske promjene, a među zabilježenim vrijednostima postoji vrlo jaka negativna korelacija (r = - 0,947). Udio masti je od 0,86 do 4,47%. Najniže vrijednosti zabilježene su u veljači, a najviše u listopadu. Postojala je statistički značajna povezanost između masti i vode na razini 99% pouzdanosti, što ukazuje da bi se funkcionalni odnos između udjela masti i vode mogao koristiti za procjenu masti na temelju analitičkog određivanje sadržaja vode.

Ključne riječi: *Engraulis encrasicolus,* kemijski sastav, sezonske promjene, udio masti