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## The Fatty Acid Profile of the Marine Cephalopod *Loligo vulgaris*

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### Abstract

Squids and their by-products are widely used as pre-spawning feeds in marine fish hatcheries. Therefore, we studied the fatty acid composition of mantle tissue, arms, fins, and gonads of the marine cephalopod, *Loligo vulgaris*, from a broodstock nutrition point of view. Docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids represented nearly half (>45%) of the total fatty acids, with DHA the most abundant fatty acid in all investigated tissues. The fatty acid profile of the gonads differed significantly from those of the mantle, arms, and fins; EPA was present in a significantly higher proportion (20%), perhaps indicating its important role in reproduction. Results indicate that *Loligo vulgaris* and its by-products can be an important nutritional component of broodstock feeds, serving as a major source of DHA and EPA.

### Introduction

Most cephalopod species are important elements in the marine food chain (Caddy, 1983). The marine cephalopod, *Loligo vulgaris*, is one of the most sought after sea foods in the Mediterranean Sea. In marine fish aquaculture, it is common to use cephalopods as broodstock feeds (Zohar et al., 1995). Most marine fish hatcheries feed squid to bass and bream broodstock during vitellogenesis and spawning. Although total body lipids represent only a small percentage of their dry weight (Navarro and Villanueva,

2000; Passi et al., 2002), saponifiable lipids contain significant n-3 fatty acids (Ferrara et al., 2001). Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are crucial nutrients for the survival and growth of eggs and larvae of most marine fish (Sargent, 1995). Zohar et al. (1995) found that including squid oil in feeds for bream (*Sparus aurata*) broodstock increased egg and larvae survival.

During processing of squid for human consumption, the tentacles and mantles are usually saved for packaging while the gonads and

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internal organs are discarded. Most studies concerning the fatty acid profiles of squid involve mantle tissues (Sinanoglou and Meimaroglou, 1998; Okuzumi and Fujii, 2000; Ferrara et al., 2001; Phillips et al., 2002). There have been few studies of the fatty acid compositions of cephalopods (Navarro and Villanueva, 2000, 2003) and seasonal changes in their fatty acid profiles (Ozyurt et al., 2006). The purpose of this study was to determine differences in fatty acids in several tissues of *L. vulgaris* as an indication of the potential use of squid by-products in marine fish aquaculture.

#### Materials and Methods

**Collection of samples.** Four specimens of *L. vulgaris* were obtained from trawlers at the local fisheries dock in Izmir in May 2005 and transferred to the laboratory on ice. Upon arrival, the specimens were weighed and the mantle length (ML) was measured. Sex and gonadal development were determined according to Lipinski (1979). Three specimens were stage 4 males (15.5 and 17.4 mm ML, 96.8 and 101.5 g) and one was a stage 4 female with a developed gonad (18.0 mm ML, 186.7 g).

**Analytical methods.** Mantle tissue, arms, fins, and gonads were preserved in dichloromethane, then 1-2 g of each were homogenized with a glass pestle. Similar amounts were placed on aluminum foil and dried at 65°C for approximately 48 h to determine the moisture content.

**Lipid and fatty acid analyses.** Total lipids were extracted according to Bligh and Dyer (1959) using dichloromethane:methanol:water (2:2:1 v/v/v; DCM). Approximately 1-2 g of tissue was homogenized with a glass pestle in a DCM mixture in three replicates. DCM was used since it is less toxic to humans than chloroform in laboratory conditions. Extracted lipids were dried under a stream of nitrogen in a glass vial. Two ml of hexane containing 2% HCl was added and the vial was sealed with a flame. Trans-sterification was carried out in boiling water for 1 h. Fatty acid methyl esters (FAME) were extracted with hexane-water partitioning and analyzed using a Fisons Instruments gas chromatography equipped

with a PSS (Split-Splitless) injector and a flame ionization detector with a J&W DB-23 (code 122-2332) column (30 m length, 0.25 mm I.D.), coated with 0.25 µm film. The injection split was 50 ml/min and helium was used as the carrier gas (70 kPa). The flame ionization detection (FID) was set at 100 kPa air and 50 kPa hydrogen pressures. The injector and detector temperatures were set at 290°C and 300°C, respectively. The temperature was set to start at 155°C (1 min) and increase at 2°C/min until reaching the final 240°C.

#### Results

The moisture contents of mantle tissue, arms, and fins were 76.1%, 76.2%, and 79.1%, respectively. The total fatty acids of the lipids in all three tissues were dominated by PUFA. DHA and EPA constituted approximately 45% of the FAME in all tissues (Table 1). Saturated fatty acids were mostly represented by 16:0 that formed almost one third of all FAME. Stearic and myristic acids were present in much lesser percentages. Mantle tissue, arms, and fins were almost identical in terms of total fatty acids. Gonads, on the other hand, contained significantly higher levels of EPA and arachidonic acid ( $p \leq 0.05$ ) and lower percentages of palmitic and docosahexaenoic acid ( $p \leq 0.05$ ).

#### Discussion

The total lipid content of many cephalopods has been documented in detail (Okuzumi and Fujii, 2000; Phillips et al., 2002; Passi et al., 2002). Total lipids in squids and cuttlefish varies 1-2 % on a wet weight basis (Okuzumi and Fujii, 2000). Phospholipids are the main components of lipid moieties of saponifiable lipids in several squid species (Passi et al., 2002), representing 62-84% of the total lipids (Okuzumi and Fujii, 2000). We found that the total fatty acids of the studied tissues are dominated by DHA and EPA. Although no other study compares the fatty acid compositions of different tissues, similar values were reported by Navarro and Villanueva (2000) and Passi et al. (2002) for *L. vulgaris*.

In *Sepia officinalis*, Sinanoglou and Meimaroglou (1998) found that the total DHA

Table 1. Fatty acid composition (%) of mantle, arms, fins, and gonad (n = 3).

Fatty acid	Mantle	Arm	Fin	Gonad
C 14:0 MA	4.4±0.5	2.5±0.06	2.4±0.18	1.7±0.18
C 15:0	0.60±0.02	0.6±0.06	0.6±0.03	0.4±0.01
C 16:0 PA	28.3±0.62	27.7±0.87	30.5±3.55	24.6±0.16
C 16:1 n7	0.9±0.08	0.6±0.13	0.6±0.07	0.4±0.09
C 18:0 SA	4.8±1.32	6.1±0.36	5.8±0.16	5.8±0.11
C 18:1 n9	4.4±1.36	2.1±0.49	2.3±0.28	1.8±0.01
C 18:1 n7	1.6±0.2	1.7±0.27	1.6±0.32	1.5±0.33
C 18:2 n6	0.2±0.02	0.3±0.13	0.2±0.09	0.3±0.04
C 20:1 n9	2.0±0.04	2.3±0.14	1.8±0.20	3.5±0.21
C 20:4 n6 AA	0.9±0.11	1.2±0.18	1.3±0.13	1.6±0.13
C 20:5 n3 EPA	14.6±0.14	15.0±0.28	14.2±0.31	20.3±0.81
C 22:1 n9	0.8±0.26	0.5±0.06	0.5±0.03	0.5±0.04
C 22:5 n6	0.3±0.03	0.4±0.05	0.5±0.04	0.4±0.03
C 22:5 n3	0.5±0.08	0.8±0.22	0.6±0.22	0.5±0.03
C 22:6 n3 DHA	32.5±2.63	33.4±0.75	30.9±1.84	26.4±2.57
C 24:1 n9	0.3±0.03	0.2±0.04	0.2±0.07	0.2±0.04
Σ Saturated FA (%)	37.2±0.54	36.8±0.53	39.5±3.60	32.6±0.45
Σ Unsaturated (%)	59.0±0.70	58.5±0.72	54.7±2.26	57.4±2.19
Unidentified (%)	3.8±1.25	3.6±0.86	5.6±2.05	9.7±1.76
Σ n3 FA (%)	47.7±2.34	49.6±0.73	46.3±2.06	47.2±1.88
Σ n6 FA (%)	1.1±0.15	1.9±0.34	1.5±0.10	1.7±0.40
Σ monoenes (%)	10.2±1.62	7.4±0.64	6.8±0.64	7.9±0.13

MA = myristic acid; PA = palmitic acid; SA = stearic acid; AA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid

content of neutral and polar lipids is 30.69% of the FAME while Ozyurt et al. (2006) found that DHA was 23.9-29.5% of the total lipids depending on the season. In our study, samples were collected in the spring when DHA seems to reach its maximum. Seasonal variations may influence fatty acid compositions. The lowest DHA level was found in the gonads, mostly of males in stage 4. Navarro and Villanueva (2003) found that total lipids contained 29.3% DHA in *L. vulgaris* hatchlings. In our study, DHA represented 26.4% of the FAME. Presumably, more DHA accumu-

lates during the late gonad development.

The EPA level ranged 14.2-20.3%. Passi et al. (2002) reported that EPA represented 14.1% of neutral lipids and 21.8% of polar lipids in *L. vulgaris*. Our values coincide with those reported by Navarro and Villanueva (2000) for *L. vulgaris* hatchlings.

Palmitic acid ranged 24.6-30.5%. Although less desired by nutritionists, palmitic acid is often abundant in marine lipids at approximately 20% of the total fatty acids of cephalopod tissues (Passi et al., 2002; Phillips et al., 2002; Ozyurt et al., 2006).

From the broodstock nutrition point of view, *L. vulgaris* can be an important source of DHA and EPA that are critical for egg and larvae development of marine fish. Hence, the by-products of squid processing has important merit in aquaculture nutrition.

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