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ORIGINAL ARTICLE

Lipid composition and antioxidant activity of liver oils from ray species living in Tunisian coasts

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Abstract The proximate composition, fatty acid profiles, physicochemical properties and radical scavenging activities of liver oil from three ray species, *Dasyatis pastinaca*, *Dasyatis violacea* and *Rhinoptera marginata*, were investigated. Lipid contents of *D. pastinaca* (58.27%) and *D. violacea* (57.33%) were significantly high compared to those of *R. marginata* (10.90%). Among minerals, K and Na were the most abundant elements and the highest values were observed for *R. marginata* (153.7 and 115.86 mg/100 g, respectively). The fatty acid profiles exhibited a dominance of unsaturated fatty acids exceeding 65% of the total fatty acids. C16:0, C18:0 and C14:0 were the major saturated fatty acids. The most abundant monounsaturated fatty acids were C18:1 (10.88–21.98%) and C16:1 (4.47–23.95%). Interestingly, omega-3 polyunsaturated fatty acid profiles exhibited a dominance of eicosapentaenoic acid (3.36–5.51%) and docosahexaenoic acid (9.07–30.50%). *D. pastinaca* contained the highest carotenoid and total phenolic content accompanied with the strongest free radical scavenging abilities. This study suggests that ray livers which were actually wasted, could be used as new raw material for omega-3 polyunsaturated fatty acid oil production and a good source of carotenoids and phenolic compounds.

1. Introduction

In the new millennium, there is a growing demand for marine fish oils, the main source of omega-3 polyunsaturated fatty acids (n-3 PUFAs) for use in human food, nutraceutics and pharmaceuticals (Bergé and Barnathan, 2005). n-3 PUFA family consists of: alpha-linolenic acid (ALA C18:3) and its longer-chain metabolites: eicosapentaenoic acid (EPA C20:5) and docosahexaenoic acid (DHA C22:6). Interestingly, health benefits of EPA and DHA are well demonstrated mainly in the prevention of cardiovascular diseases, lipotoxicity, human breast cancer, inflammatory diseases, asthma and Alzheimer's disease (Martinez et al., 2010). Due to the general decline of fish stocks, seafood should be used as good as possible

Abbreviations: SFAs, saturated fatty acids; UFAs, unsaturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; n-3 PUFAs, omega-3 polyunsaturated fatty acids

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involving the investigation of fish processing by-products and low value fishes (Kacem et al., 2011; Shen et al., 2007; Bergé and Barnathan, 2005).

Along the Tunisian coast various fish species are caught for their fins; the head and viscera including the liver are discarded. However, only a few detailed reports on lipid and fatty acid content of by-products and underutilized fishes from this area are available. Recently, viscera of *Sardinella aurita*, *Sarpa salpa* and *Sepia officinalis* from these waters were investigated indicating a high level of n-3 PUFAs, up to 20% including EPA and DHA as major components (Kacem et al., 2011). Among the Tunisian underutilized fishes, the ray species (belonging to the subclass of elasmobranchii) constitute an attractive marine product, because of their abundance and lower price. It seems therefore interesting to propose the utilization of the ray liver as a source of high quality oil suitable for human consumption. Up to now, there are no studies of lipids and fatty acid composition carried out on ray species from the Tunisian coasts. Nevertheless, scientific information on the composition of elasmobranch liver oil was obtained mainly for shark species (Hayashi and Kishimura, 2000). However, only little information dealing with liver oil of ray species caught in different areas was reported. For example, fatty acid distributions in the muscle, liver, and gonads of three ray species (*Dasyatis marmorata*, *Rhinobatos cemiculus*, and *Rhinoptera marginata*) from the east tropical Atlantic Ocean were determined by Ould El Kebir et al. (2003). Also, liver lipids of *Dasyatis brevis* and *Gymnura marmorata* from the California Gulf (Navarro-Garcia et al., 2004) and *Himantura bleekeri* from the Indian coast (Le Nechet et al., 2007) were analyzed. These authors had found that the liver lipidic fractions of all ray species studied contained a high amount of PUFAs (up to 30% of the total lipids), mainly composed of DHA and EPA. Hence, the objective of this work was to characterize the liver oil of three commercial ray species *Dasyatis pastinaca*, *Dasyatis violacea* and *Rhinoptera marginata* from the Mediterranean Sea (Gabes Gulf of Tunisia).

2. Experimental

2.1. Biological materials

Livers of three ray species (*D. pastinaca*, *D. violacea* and *R. marginata*) caught from the east coast of Tunisia (Gabes Gulf), during January 2009 were used in this work. Fresh fishes were purchased from the local market of Sfax city and livers were isolated and kept frozen (−20 °C) prior to analysis.

2.2. Proximate composition analysis

Dry matter was determined by oven-drying at 105 °C to constant mass. Crude proteins were analyzed according to the Kjeldahl method (AOAC, 1990). A factor of 6.25 was used for the conversion from total nitrogen to crude protein. Fat content was determined according to a method described by Folch et al. (1957). The ash content was determined by combustion of the sample at 550 °C for 8 h. Then, mineral constituents (K, Na, Mg Ca, Fe, Cu, Zn, and Mn) were analyzed separately using an atomic absorption spectrophotometer (Hitachi Z6100, Japan). The density, iodine index and saponification index, were determined following the methods 920.213, 993.20 and 921.160, respectively of the AOCS (AOCS, 1993).

2.3. Fatty acid composition

To determine fatty acid composition, lipids were dissolved in 0.5 ml of hexane. Then, 0.2 ml of potassium hydroxide in methanol (2 N) was added for the fatty acid methylation process. The mixture was vortexed, centrifuged and the upper phase containing fatty acid methyl esters was subjected to the GC/MS analysis. A Perkin-Elmer-Clarus-500 gas chromatograph equipped with HP-5MS capillary column (30 m × 0.25 mm internal diameter, film thickness 0.25 μm; Hewlett–Packard) and coupled to a HP 5972A masse-selective detector (Hewlett–Packard, Palo Alto, CA, USA) was used. The column temperature was programed at 50 °C for 1 min, then the temperature was increased progressively to 250 °C (7 °C/min) and was maintained during 5 min. The temperature of the injector port was held at 250 °C (split ratio: 1/100) and the temperature of the detector was set at 280 °C. The mass spectrometer conditions were as follows: ionization voltage, 70 eV; ion source temperature, 150 °C; electron ionization mass spectra were acquired over the mass range 50–550 Da. The carrier gas was helium (99.995% purity) with a flow rate of 1.2 ml/min and the analyzed sample volume was 2 μl. The individual constituents showed by GC were identified by comparing their mass spectrometry with standard compounds of Willey libraries. Results, which are means of triplicates, were expressed as w/w percentage of total fatty acids.

2.4. Carotenoid content

The total carotene analysis in the liver oil was carried out according to Simpson and Haard (1987). The weighed oil samples were dissolved in hexane and the absorbance at 468 nm was recorded using a UV–Vis spectrophotometer (Secomam, Uvi Light XT 5). Olive oil was used as a control.

2.5. Total phenolic content

Total phenolic content was determined by the Folin–Ciocalteu method (Gutfinger, 1981). A 2.5 g sample of liver oil was extracted with 5 ml of hexane. Then 5 ml of methanol/water (60:40, v/v) was added and the mixture was vortexed vigorously for 2 min. The Folin–Ciocalteu reagent (0.5 ml) and 4.8 ml of bi-distilled water were added to the phenolic fraction. Then 1 ml of sodium bicarbonate (35%, w/v) was added and water was used to bring the final volume to 10 ml. The mixture was incubated for 2 h in the dark at room temperature and then the absorbance of the mixture was measured at 725 nm. Results were expressed in milligram gallic acid equivalents per kilogram of oil (mg GAE/kg oil). Olive oil was used as a control.

2.6. DPPH· radical scavenging assay

Free radical scavenging activity of the studied liver oil was determined using the DPPH method proposed by Lee et al. (2007). The conventional DPPH method uses methanol as solvent, which does not dissolve oil. Isooctane was selected as a

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