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Nutritional value of the Tunisian mussel *Mytilus galloprovincialis* with a special emphasis on lipid quality

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Docosahexaenoic acid (PubChem CID: 445580)
Eicosapentaenoic acid (PubChem CID: 446284)
Myristic acid (PubChem CID: 11005)
Omega-3 Fatty Acids (PubChem CID: 56842239)
Omega-6 Fatty Acids (PubChem CID: 56842208)
Palmitic acid (PubChem CID: 985)

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ABSTRACT

This study reports, for the first time, data on nutritional quality parameters in Tunisian mussels, *Mytilus galloprovincialis*, with an especial emphasis on lipid compounds. Mussel condition index (CI), proximate composition and fatty acid profiles were investigated for a one year period in order to identify the best harvesting period. Analysis revealed that polyunsaturated fatty acids (PUFA) were the dominant fatty acids with a prevalence of n-3 over n-6 forms. Pearson's correlation indicated a strong relationship between CI and PUFA compound and Principal Components Analysis suggested that, from winter to summer, the product maintained a condition sufficiently good for marketing. The best CI and lipid quantity/quality occurred during summer and this may be used as criteria for product labelling. The study also included a thorough literature review that allowed data comparison on mussels from various Mediterranean sites and allowed the mussels from the Bizerte lagoon (North of Tunisia) to be differentiated.

1. Introduction

Lipids in human diets have several important roles including the provision of a significant proportion of the body's necessary energy requirements, as cell membrane components containing lipophilic vitamins, and being the source of essential fatty acids (Pigott & Tucker, 1987). Several studies, however, have revealed negative effects of highly saturated fatty acids lipid consumption cardiovascular pathologies (Valfré, Caprino, & Turchini, 2003; Wu, Dyer, King, & Innis, 2013). Studies of human dietary habits have revealed the health benefits of seafood consumption; bivalve molluscs (e.g. oysters and mussels) are considered to be highly nutritive and attract an increasing demand in international markets (Pogoda, Buck, Saborowski, & Hagen, 2013). Shellfish display interesting nutritional characteristics as they are a rich source of proteins, carbohydrates and minerals (Bongiorno et al., 2015) and provide an almost unlimited variety of fatty acids with beneficial roles in human health (Ackman, 1989).

The dietary human health benefits of mussels are a reflection particularly of their fatty acid compound contents [e.g. omega-3 polyunsaturated fatty acids: docosahexenoic acid (DHA C22:6-3) and eicosapentaenoic acid (EPA, C20:5n3)] [(Bongiorno et al., 2015; Grienke, Silke, & Tasdemir, 2014)], which cannot be synthesised by humans and must be obtained from the diet (Alasalvar, Taylor, Zubcov, Shahidi, & Alexis, 2002). However, Baek et al. (2014) have shown that these nutritional attributes of bivalves are both seasonally and geographically variable [(e.g. the lipid and fatty acid contents of *M. galloprovinialis* fluctuate as a result of the synergistic interaction between the quality and quantity of their diet and reproductive cycle)] (Fernández et al., 2015). Such fluctuating attributes determine the quality of the shellfish as products for processing or labelling.

Given such interspecific and intraspecific, geographical and seasonal variation in the fatty acids distribution in mussels, the present study was designed to examine the composition of the mussels harvested from the lagoon of Bizerte, Southwestern Mediterranean Sea. It should be noted that, since the 1960′s, Tunisia has harvested shellfish exclusively in the Bizerte (Occidental Mediterranean Sea) lagoon where farming methods are controlled. Such production is not, however, well advertised in terms of the development of an export market for the products. However, there remains the opportunity to develop such a market within the context of the global seafood trade.

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Consequently, the aims of this study were to assess the seasonality of the condition index, nutritional parameters and fatty acids content of the commercial mussel *Mytilus galloprovincialis* in the Tunisian fisheries and to evaluate the nutritional values of lipid and fatty acids quality of these products. It was also intended to rectify the noticeable gap in the literature on lipid physiology in marine invertebrates from the South-western Mediterranean Sea and to provide scientific information for future, data comparisons within the context of other case studies or hypotheses. Such data on the optimisation of mussel culture conditions optimisation or product labelling are of high interest to local farmers.

2. Materials and methods

2.1. Sample collection and pre-treatment

During a period of 12 months, monthly samples of the mussel, *Mytilus galloprovincialis*, of commercial size (mean length of 60.72 ± 0.21 mm), were brought to the laboratory after purification in clean seawater. In the laboratory, mussels were cleaned and then divided on the basis of condition index and biochemical determinations. The soft tissues of 6 pooled lots (n = 20 animals in each case) were homogenised and stored immediately at −80 °C until analyses.

2.2. Condition index determination

Approximately one kg (mean of *ca* 50 specimens) was taken randomly for the determination of biometric parameters. The mean length of analyzed specimens was 60.72 ± 0.21 mm. After the determination of individual total weights, mussels were cooked in the microwave at maximum temperature power level for 2 min and cooked meat was weighed. Condition index was calculated according to the Davenport & Chen (1987) formula:-

$$CI = \frac{Cooked\ meat\ weight}{Cooked\ meat\ weight + Shell\ weight} \times 100$$

2.3. Poximate composition determination

Each month the homogenized samples of soft tissues were subjected to a number of physical and biochemical analyses. Moisture and ash evaluations were made according to AOAC (1997) method; moisture content (method 950, 46) was determined by drying sample to constant weight at 103 °C ± 2 °C, and ash content by incineration in a muffle furnace at 550 °C (method 920, 153). Total protein content was determined using the modified Lowry method (Hartree, 1972) with a maximum at absorption λ = 650 nm using a μQuant™ microplate spectrophotometer (BioTek). Total carbohydrate content was performed using the phenol sulphuric acid method as described by Dubois, Gilles, Hamilton, Rebers, & Smith (1956) with a maximum at absorption λ = 490 nm using a LLG-UniSPEC 2 Spectrophotometer. Total protein and carbohydrate values were calculated using Bovine serum albumin (BSA) and glucose standard calibration curves respectively.

Total lipid extraction from 1 g homogenised tissue samples was performed according to the method described by Folch, Lees, and Stanley (1957) using a chloroform: methanol (2:1 v/v) extraction solution containing 0.01% butylated hydroxytoluene (BHT) as antioxidant. After centrifugation (4000*g*, 4 °C, 20 min) the lower phase (chloroform) was removed carefully with a Pasteur pipette and the solvent was evaporated to dryness. Fats were determined gravimetrically.

2.4. Fatty acids content determination

Lipid extracts were *trans*-esterified according to the Christie (1993) method. A solution of 2% concentrated sulphuric acid in methanol was used to prepare fatty acids methyl esters (FAMEs). The Reaction was carried out overnight at 50 °C. FAMEs were separated and quantified using a gas chromatograph (Agilent/6890L) equipped with a flame ionization (FID) detector. Each extract (1 μl) was directly injected in a split ratio 25:1 into a capillary column (HP-INNOWAX, 30 m × 0.25 mm × 0.25 μm). Nitrogen was used as the carrier gas using a constant flow of 1 ml/min. The initial oven temperature of 150 °C was maintained for 2 min, raised from 150 °C to 200 °C at the rate of 15 °C/min, and then increased from 200 to 240 °C at a rate of 2 °C/min. Analysis time was 30 min for each sample. Detector and injector temperatures were 275 and 220 °C, respectively.

Chromatograms were acquired and processed using the GC Agilent Rev.B.04.03, ChemStation software and individual methyl esters, including C20:5n3 (EPA) and C22:6n3 (DHA), were identified by comparison with known standards (PUFA No.3, from Menhaden oil, SUPELCO). Repetitive injections of standard solutions were carried out to assess the analytical accuracy. The relative fatty acid results were expressed as % of the total fatty acids.

2.5. Thrombogenicity and atherogenicity indices

The equation of thrombogenicity index (IT) and atherogenicity index (IA) were calculated according to Ulbricht & Southgate (1991):-

- Index of atherogenicity (IA):

$$IA = \frac{C12{:}\ 0 + C14{:}\ 0 + C16{:}\ 0}{\sum PUFAn{-}6 + \sum PUFAn{-}3 + \sum MUFA}$$

where PUFA = Polyunsaturated fatty acids and MUFA = Monounsaturated fatty acids.

- Index of thrombogenicity (IT):

$$IT = \frac{C14{:}\ 0 + C16{:}\ 0 + C18{:}\ 0}{(0.5{*}PUFA_{n-6}) + (3{*}PUFA_{n-3}) + (0.5{*}MUFA) + \left(\frac{PUFA_{n-3}}{PUFA_{n-6}}\right)}$$

where PUFA = Polyunsaturated fatty acids and MUFA = Monounsaturated fatty acids.

For this study, the resulting data were compared with data found following an intensive survey of the last 15 years' literature pertaining to *M. galloprovincialis* from the Mediterranean basin and in which the nutritional indices of interest (e.g. n-3, n-6, EPA and DHA) were included. Comparison was based on published data using similar method of lipid extraction (Bligh and Dyer (1959), Folch et al. (1957) and fatty acid methylation and analysis using GC).

2.6. Statistics

The significance of seasonal differences in condition index and of lipid and fatty acid composition was determined using ANOVA one-way test and SPSS version 11.01 software (SPSS Chicago, IL, USA). Verification of the normality of distributions and the homogeneity of variances was made using the Kolmogorov-Smirnov test. Differences were then analyzed using Tukey test. Correlations between mussel condition index and quantitative/qualitative lipid contents were examined by Pearson's correlation coefficients using SPSS 17.0. Obtained results were also submitted to principal component analysis (PCA) using XLSTAT Version 2014.5.03, for a better visualization of seasonal patterns for condition index, lipid and fatty acids content.

3. Results and discussion

3.1. Condition index

The determination of the condition index parameter in bivalves such as *M. galloprovincialis* is of biological and technological interest. Several authors have highlighted the influence of temperature,