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ORIGINAL ARTICLE

Nutritional quality traits of raw and cooked Ark shell (Bivalvia: Arcidae): balancing the benefits and risks of seafood consumption

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Abstract Biochemical composition and fatty acid profile of raw Ark shells (RA) were compared to Ark shells submitted to three different cooking methods (BA: baking in the oven; PF: pan-frying in butter and MW: cooking in a microwave). Moisture (%) was significantly higher in RA (79.66) with respect to PF (65.09), BA (48.63) and MW (47.02). Protein (mg/g of flesh) decreased significantly from 18.62 in RA to 15.40 in MW, 13.76 in PF and 13.33 in BA. However, lipids significantly increased in MW (43.32 mg/g of flesh) and PF (63.63 mg/g of flesh) with respect to RA (35.05 mg/g of flesh). Pan-frying affected considerably triacylglycerol (TAG) and the fatty acid composition (FA) of Ark shell flesh. The most changes occurred in saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid fractions after this process. The n-3 PUFA decreased significantly from RA (16.40 mg/g dry weight) to PF (10.02 mg/g DW). While, the opposite trend was observed for n-6 PUFA, revealing that this cooking method had considerable effects on the nutritional characteristics of this edible shellfish. The analysis of lipid peroxidation markers such as thiobarbituric acid reactive substances, free fatty acid and peroxide value confirmed that both heat treatment and time of cooking caused lipid degradation, which had been more accentuated during pan-frying treatment. For the populations who consume Ark shells occasionally or frequently, baking and microwave cooking could be then considered as wiser and healthier cooking methods since they conserve better the nutritional value of this marine product. The present study will be of practical value from a health perspective for Mediterranean populations.

Keywords Ark shells · Cooking procedure · Proximate composition · Fatty acids · Triacylglycerol · Lipid peroxidation

Introduction

The Noah's ark, *Arca noae* Linnaeus, 1758 (Bivalvia: Arcidae), is one of the most important commercially exploited bivalve species in the Mediterranean sea. It is an epifaunal bivalve of hard substratum whose distribution ranges from the eastern Atlantic Ocean, the Mediterranean and Black Seas to the West Indies (Bejaoui et al. 2019a). It lives attached with a solid byssus on rocks and shells occurring either solitarily or in clumps of conspecifics with the similarly byssally attached mytilid *Modiolus barbatus* at depths ranging from approximately low tide level to deeper than 100 m. Due to its high commercially importance as a seafood resource, *A. noae* is harvested by local fishermen and scuba divers in many Mediterranean countries (Župan et al. 2012). Since 1940s, *A. noae* was a major target species of traditional fisheries. In Greek coasts, the mean annual production of *A. noae* was over 6ts for the fishing periods from 2000 to 2004 for the local fishermen in the Gulfs of Kalloni and Geras (Paspatis and Maragkoudaki 2005). Furthermore, *A. noae* harvested from Croatian coasts had, over decades, a relatively high and stable price on the market, it is consumed by the local population and

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even sold on the black market during the tourist season (Župan et al. 2012). In support of sustainable aquaculture and exploitation of natural stocks, many authors conducted a series of scientific researches to investigate the possibilities of *A. noae* introduction into aquaculture. The potential of Ark shell for aquaculture was assessed through analyzing their survival, feeding and growth rates (Kozul et al. 2011; Peharda et al. 2013). Župan et al. (2014) investigated survival, growth, condition index and stable isotope composition of *A.* noae under experimental aquaculture conditions and in the natural habitat.

Among the thousands of species used directly or in processed form, seafood provides a wide variety of products for human nutrition (Oehlenschlager 2012). Last decades, the culinary art of cooking shellfish have evolved and presented new cooking methods that are now used by specialized chefs worldwide and even by an ordinary cook. Most shellfish can be cooked using any of the basic techniques for fish and this simply by following the same guidelines. Indeed, the delicate nature of seafood requires extra care and attention during the cooking process because it is easy to overcook and ruin their flavors and textures. Therefore, when cooking seafood, in particular bivalves, it is well recommended to adjust the temperature and time of cooking to better preserve its nutritional value (Ghribi et al. 2017; Bejaoui et al. 2019a). Despite there being so many methods for cooking seafood (baked, steamed, grilled, fried, canned…etc.), some are quick, some are healthy and some are just simply easy. However, the question that arises: which one is the best for preserving both flavors and good nutritional quality? For years, several studies have focused only on the nutritional quality of raw shellfish (Ghribi et al. 2018; Bejaoui et al. 2019b). However, studying cooked shellfish is also of greater interest because (i) they have been the perfect ingredient for many dishes like soups, salads, pasta, pizza etc. and (ii) recent papers have demonstrated that mostly young people preferred to eat cooked shellfish with respect to the raw products (Masson et al. 2016). There is already a good public awareness about the benefits of eating seafood as consumers have become increasingly vigilant about their health with regard to the consumption of red and white meat. However, there is still a lot of concern arising from reports about the potential risks of consuming seafood (Oehlenschlager 2012).

It is important to understand how the components of seafood (proteins, lipids, carbohydrates and water) react when heated. In fact, seafood supplies the human body with a sufficient amount of benefits. Nowadays, a lot of cooking methods are applied (boiled, pan-fried, deep-fried, grilled, baked, steamed, cooked in micro-wave…). Each of them has its advantages and disadvantages. In this regard, the present study seeks to find the most suitable cooking method to consume the Ark shells while keeping their flavors and good nutritional intake. Despite the high commercial importance of *A. noae* in the Mediterranean, information about different cooking methods of Ark shells remains very scarce (Ghribi et al. 2017) and concerns other species like oysters, mussels and clams (Bejaoui et al. 2019a; Felici et al. 2020). To provide useful information on the nutritional traits of cooked Ark shells required for its consumption, either by local or foreign consumers, this study was performed considering three culinary treatments: (BA: baking in the oven; PF: pan-frying in butter in a pan and MW: cooking in a microwave). The effects of the cooking preparations were evaluated on proximate composition, triacylglycerol and fatty acid profile of Ark shells harvested from the Tunisian coastal lagoon (Bizerte lagoon). The lipid peroxidation process was also investigated in cooked Ark shells through three markers: Thiobarbituric acid reactive substances (TBARS), free fatty acid (FFA) and peroxide value (PV). Such a report will be of great importance since this has been rarely investigated before.

Materials and methods

Sample preparation

Mature Ark shells of commercial size (length: 55.89 ± 5.56 mm; total weight: 31.39 ± 4.78 g) were collected by a scuba diver from the Bizerte lagoon (North of Tunisia; 37°09′03″N, 9°52′03″E). Specimens were kept in a cool box and directly transported to the laboratory. All epibiotic materials were removed from the shells and Ark shells were carefully opened using scalpel (byssus excluded), washed with running water and drained by absorbent paper. Then, they were divided into four homogeneous groups subjected to different culinary preparation: raw (RA, as Control, 10 animals), baked (BA, 10 animals), cooked in a microwave (MW, 10 animals) and pan-fried in butter (PF, 10 animals). The four groups of Ark shells were removed from their shells. BA samples were prepared by cooking the Ark shells at 170 °C for 8–10 min in a preheated electric oven. MW Ark shells were placed in a single layer in a microwave safe dish. 1/4 cup of hot water was added to the dish and then placed in the microwave (LG MS-2644B). Ark shells were cooked on high for about 4–5 min at 2450 MHz. Samples are considered ready when the shells open wide and the flesh, subsequently, easily detaches from the shell. PF Ark shells were fried in margarine [Local mark, 5 g (∼ 1 tsp) for each sample; total saturated fatty acids SFA: 32.3 ± 4%, total monounsaturated fatty acids (MUFA): 28.2 ± 5.1%, total polyunsaturated fatty acids PUFA: 22.8 ± 3.6%, PUFA n-3

1.9 ± 0.1% and PUFA n-6 20.3 ± 2.3% of total fatty acids (FA); Table 1] for 4 min at 180 °C (2 min each side). After cooking, PF samples were placed for 3 min on absorbent paper towels. During this process, professional pans (French cookware Tefal) were used. During all cooking treatments, sample internal temperature was checked permanently using a food digital thermometer to prevent overcooking and to ensure food safety. Samples were homogenized with a blender and stored at − 20 °C until further analysis. All the culinary preparations steps were realized based on previous studies and inspired by a professional cooking book (Bejaoui et al. 2019a; Felici et al. 2020).

Proximate composition

According to AOAC (2005), moisture (%) was determined after heating the sample at 110 °C for 24 h by weight difference between initial and final sample tissue mass. Protein (mg/g of flesh) content was extracted and determined using bovine serum albumin (BSA) as standard following the method described in Lowry et al. (1951). Lipids (mg/g of flesh) were extracted using the solvent mixture chloroform–methanol (2:1, v/v) according to the method of Folch et al. (1957). All the biochemical compounds investigated in this study on the cooked and uncooked Ark shell samples were measured in ten replicates.

Table 1 Fatty acid composition of margarine before and after pan-frying

FA (mg/g)	Before	After
C14:0	2.125 ± 0.134	2.678 ± 0.151
C15:0	0.156 ± 0.026	0.197 ± 0.012
C16:0	25.470 ± 1.330[a]	19.320 ± 0.152[b]
C16:1	0.306 ± 0.012	0.299 ± 0.017
C16:2	0.298 ± 0.065	0.275 ± 0.051
C16:3	0.097 ± 0.010	0.102 ± 0.010
C16:4	0.144 ± 0.022	0.155 ± 0.02
C18:0	4.56 ± 0.623[a]	3.001 ± 0.177[b]
C18:1	27.861 ± 4.365[a]	20.111 ± 2.44[b]
C18:2n-6	19.876 ± 2.978[a]	14.320 ± 0.754[b]
C18:3n-6	0.456 ± 0.034[a]	0.235 ± 0.019[b]
C18:3n-3	1.987 ± 0.132	1.566 ± 0.144
SFA	32.311 ± 3.921[a]	26.197 ± 2.401[b]
MUFA	28.166 ± 5.122[a]	20.410 ± 2.456[b]
PUFA	22.858 ± 3.625[a]	16.649 ± 1.562[b]
PUFA n-3	1.987 ± 0.132	1.566 ± 0.144
PUFA n-6	20.332 ± 2.310[a]	14.555 ± 0.879[b]

Values are mean ± SD, n = 3

Different letters indicate significant differences at $p < 0.05$

Fatty acid analysis

Following the method of Cecchi et al. (1985), the lipid extracts of uncooked and cooked Ark shells were transesterified using as an internal standard: the nonadecanoic acid (C19:0; Sigma). This standard is essential for the quantification of Ark shell fatty acids. After the extraction of fatty acid methyl esters (FAMEs) with hexane: diethyl ether (1:1, v/v) solution, they were separated by gas chromatography (HP, 6890 GC) equipped with a split/splitless injector and a flame ionization detector. An Innowax capillary column (30 m, 250 µm internal diameter/0.5 µm film thickness) was used and the temperature was programmed to increase from 50 to 180 °C at a rate of 4 °C/min. The carrier gas used was Nitrogen. The injector and detector temperatures were 250 °C and 275 °C, respectively. The temperature ramp from 50 to 180 °C at a rate of 4 °C/min, followed by an increase of 40 °C min^{-1} to 220 °C to stabilize at this temperature for 7 min. The identification of FA was performed in comparison with a mixture of commercial standards methyl esters (SUPELCO 47085U PUFA No: 3). Fatty acid peaks were analyzed and integrated using HP chemstation software and expressed as mg/g DW.

Triacylglycerol analysis

The lipid classes were separated using thin-layer chromatography (TLC) silica plates (20 × 20 cm, silica gel 60, Merck, Germany) following the method of Olsen and Henderson (1989). To separate the TAG fraction, 400 µl of total lipid extracts (TL) were developed on the silica plates with neutral lipids solvent [methyl acetate, isopropanol, chloroform, methanol, and 0.25% KCl (25:25:25:10:9, v/v)]. The TAG fraction was then visualized under UV light (plate reader; UV transilluminator MUV21) after spraying with 0.1% 2′-7′dichloro-fluorescein in absolute methanol.

Nutritional quality indices

The nutritional quality of the lipid fraction of raw and cooked Ark shells samples was determined by examining the fatty acid profile and taking into consideration three nutritional quality indices (NQI):

(a) The n-3/n-6 PUFA ratio and $\sum$ (EPA + DHA) were calculated as described by Marques et al. (2010) and Unusan (2007).

(b) Atherogenicity Index (AI) AI = [(4 × C14:0) + C16:0]/[n-6PUFA + n-3 PUFA + ∑MUFA] (Ulbricht and Southgate 1991).
(c) Thrombogenicity Index (TI) TI = [C14:0 + C16:0 + C18:0]/[(0.5 × MUFA) + (0.5 × n-6 PUFA) + (3 × n-3 PUFA) + n-3/n-6 PUFA] (Turan et al. 2007).

Lipid degradation

Each whole animal soft tissue (n = 10) per condition was homogenized in cold condition with an ultra Turrax® in phosphate buffer (0.1 M, pH = 7.4), then centrifuged at 9000×*g* for 20 min at 4 °C. The supernatants of the homogenized tissues were used for lipid biomarker assays.

Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances can be detected by a colorimetric reaction with thiobarbituric acid (TBA) by the method of AOCS (1989). The detection of TBARS present in biological samples is based on the reaction in which 2 molecules of TBA react with an MDA molecule and cause the formation of a red chromogen. 0.5 ml of the supernatant was placed for one hour in a water bath at 37 °C. Then, 0.5 ml of trichloroacetic acid (TCA 30%) and 0.5 ml of H_2O were added to the solution. After stirring the mixture and centrifugation at 3500 rpm for 10 min, 1 ml of supernatant is removed and 1 ml of TBA (0.67%) is added. The mixture was placed in a boiling water bath (100° C) for 10 min. Absorbance was measured at 532 nm. The level of TBARS is expressed in mg of MDA/kg of flesh.

Peroxide value (PV)

The peroxide value representing the concentration of hydroperoxide (the primary oxidation products) was determined following the method of AOCS (1989). An aliquot of 0.5 ml supernatant was mixed with 5 ml of chloroform and glacial acetic acid (0.2 ml). The reaction was initiated, and a yellow coloration was obtained, after adding a saturated potassium iodide solution. The first mixture was titrated with sodium thiosulfate solution (0.1 N) after adding 1 ml of the starch solution (blue coloration). PV was calculated according to Eq. (1):

$$\mathrm{PV} = (\mathrm{S} - \mathrm{B}) \times \mathrm{N\ thiosulfate} \times 1000/\mathrm{w} \quad (1)$$

where, S is the sample titration, B is the blank titration and w is the weight of the sample.

Free fatty acid (FFA)

The amount of FFA was determined as described in AOCS (1989). Approximately, we homogenized 1 ml of samples supernatants with 5 ml of absolute ethanol and phenolphthalein. The mixture was then vortexed for 10 min under hot temperature (50–60 °C). FFA amount was obtained under the appearance of pink color by titration with KOH solution (0.1 N). FFA was calculated following Eq. (2):

$$\mathrm{FFA} = (\mathrm{Te} - \mathrm{Tb}) \times \mathrm{N} \times \mathrm{M}/\mathrm{w} \quad (2)$$

where, Te is the sample titration, Tb is the blank titration, N is the normality of ml of KOH solution used, M is the molar weight of KOH, and w is the weight of the sample.

Statistical analysis

The software STATISTICA 8 (Stat-Soft Inc,) was used to analyze all the data. The results are given as means ± standard deviations (SD). The homogeneity and the normality of variables were checked using the Shapiro–Wilcoxon test. The one-way analysis of variance (ANOVA) followed by a posthoc Tukey's test ($p < 0.05$) was conducted to determine differences among the means of different variables in raw and cooked samples at a significance level of 5% ($p < 0.05$). The application of the principal component analysis (PCA) was essential to determine the relationship between biochemical variables in the four groups of Ark shells (RA, BA, PF and MW).

Results

Biochemical composition of raw and cooked Ark shell

Significant differences in the proximate composition of raw and cooked Ark shells (Table 2) were observed. Our results indicated a significant decrease in the moisture content (%) of all cooked specimens reaching 38% and 40% of reduction for BA and MW, respectively, when compared to RA ($p < 0.05$). Lipid content increased significantly ($p < 0.05$) in PF (63.63 ± 13.48 mg/g of flesh) and MW (43.32 ± 10.08 mg/g of flesh) compared to RA (35.05 ± 15.17 mg/g of flesh) and BA (32.00 ± 13.6 mg/g of flesh). However, the protein content was significantly lower in BA and PF samples (16 ± 2.85 and 13.76 ± 2.53 mg/g of flesh, respectively; $p < 0.05$) compared to RA and MW Ark shells (18.62 ± 2.93 and 18.48 ± 3.06 mg/g of flesh, respectively).

Table 2 Proximate composition of raw and cooked Ark shell

Proximate composition	RA	BA	MW	PF
Moisture(%)	79.66 ± 0.82[a]	48.63 ± 4.80[b]	47.02 ± 7.76[b]	65.09 ± 4.17[b]
Lipid (mg/g of flesh)	35.05 ± 15.17[a]	32.00 ± 13.59[a]	43.32 ± 10.08[b]	63.63 ± 13.48[b]
Protein (mg/g of flesh)	18.62 ± 2.93[a]	16 ± 2.85[b]	18.48 ± 3.06[a]	13.76 ± 2.53[b]

Values are mean ± SD, n = 10

Different letters indicate significant differences at $p < 0.05$ between different cooking processes

Changes on fatty acid profile of raw and cooked Ark shell

FA composition of cooked and raw *A. noae* total lipid is summarized in Table 3. Palmitic acid (C16:0) was the prominent saturated fatty acids (SFA) in all samples significantly higher in PF (103.3 mg/g DW) than in the RA Ark shell (17.54 mg/g DW). Stearic (C18:0) and myristic (C14:0) acids were the second and the third most representative SFA. Although, recorded in lower proportions than C16:0, the C14:0 and C18:0 SFA showed a significant increase in PF Ark shell (18.81; 12.07 mg/g DW) with respect to RA (5.07; 3.42 mg/g DW). As consequence, the total of SFA remained stable for BA and MW Ark shell, while it showed a remarkable increase in PF as compared to RA. Among MUFA, the most prevalent was oleic acid (C18:1) with proportions 16 up fold in PF (80.9 mg/g DW) with respect to RA (5.35 mg/g DW). Palmitoleic acid (C16:1) was the second most important MUFA without significant differences between PF (2.83 mg/g DW), MW (2.58 mg/g DW), BA (3.13 mg/g DW) and RA (3.96 mg/g DW). Consequently, the MUFA increased significantly from the raw sample RA (11.9 mg/g DW) to the pan-fried sample PF (86.25 mg/g DW). As regards PUFA n-3, the DHA (C22:6n-3) showed the highest proportion in all samples with a significant decrease in PF (1.71 mg/g DW) compared to RA specimens (5.84 mg/g DW), while it remains stable for BA (5.94 mg/g DW) and MW samples (6.62 mg/g DW). Meanwhile, no significant difference was observed in the proportion of eicosapentaenoic (EPA) between RA, BA, MW and PF. Total n-3 PUFA showed a significant reduction of 38% from RA (16.40 mg/g DW) to PF (10.02 mg/g DW). However, the two cooking treatment BA and MW did not show any significant difference ($p < 0.05$) with RA samples. Among the n-6 PUFA, linoleic acid (C18:2n-6) was prevalent in all samples. However, its proportion increased significantly only in PF treatment (53.73 mg/g DW). No significant changes in terms of PUFA n-3, PUFA n-6 and the sum of PUFA were signaled in the BA an MW cooking treatment compared to RA specimens in which PUFA n-3 dominated PUFA n-6. These facts did not correspond to the results found in PF treatment.

Nutritional quality indices (NQI)

NQI are presented in Table 3. The proportion of EPA + DHA, for BA and MW, was constant with respect to RA Ark shells. While, for n-3/n-6, a significant reduction was observed after all cooking methods and was more pronounced in PF samples (Table 3). Overall, EPA + DHA and n-3/n-6 indices decreased significantly by 71% and 93%, respectively, in PF Ark shells. While, TI was 2.6 folds higher in PF Ark shells than the RA samples. When to the AI index, a similar trend was recorded in all cooked Ark shells and did not differ from RA.

Changes on triacylglycerol (TAG) composition of raw and pan-fried Ark shell

For this study, we choose to report the TAG composition of RA and PF Ark shells since the major changes in total lipids occurred for PF samples. The amount of triacylglycerol (TAG) varied significantly after pan-frying (Table 4). Our results indicated a significant increase in SFA proportion ($p < 0.01$), mainly for C16:0 and C17:0, which increased by 134% and 213%, respectively. While, C18:0 decreased by 96% as compared to the raw tissues. The comparison between RA and PF Ark shells revealed a significant increase of MUFA by 411% especially for C15:1, C16:1, C18:1, C20:1 and C24:1 ($p < 0.05$). As regards PUFA, significant increases were also observed in n-6 PUFA (76%), EPA (654%), C21:5 (451%), C22:2i/2j (161%), C20:4n-3 (44%), C20:3n-3 (95%), C20:4n-6 (54%) and C18:2n-6 (80%). However, no significant changes were observed for DHA and n-3 PUFA when compared to RA Ark shells.

Lipid peroxidation

The TBARS, PV and FFA levels in the raw and cooked samples were presented in Fig. 1. TBARS, PV and FFA levels increased significantly in BA and MW ($p < 0.05$) when compared to RA. However, the most changes in TBARS, PV and FFA occurred in PF Ark shells with an increase of 66%, 317% and 445%, respectively.

Table 3 Fatty acid composition (mg/g DW) and nutritional quality indices (NQI) of the raw (RA), backed (BA), microwave (MW) and pan-fried (PF) Ark shells

Fatty acids	RA	BA	MW	PF
C14:0	5.07 ± 3.67[a]	3.45 ± 0.40[a]	2,69 ± 0.86[a]	18.81 ± 1.25[b]
C15:0	0.52 ± 0.39	0.61 ± 0.10	0.78 ± 0.27	0.54 ± 0.22
C16:0	17.54 ± 9.94[a]	19.26 ± 4.12[a]	19.96 ± 3.82[a]	103.30 ± 13.98[b]
C17:0	1.23 ± 0.83	1.61 ± 0.30	1.75 ± 0.40	0.99 ± 0.49
C18:0	3.42 ± 2.46[a]	4.54 ± 0.87[a]	4.78 ± 1.42[a]	12.07 ± 1.07[b]
C20:0	0.11 ± 0.13	0.06 ± 0.02	0.09 ± 0.04	0.53 ± 0.10
C22:0	0.15 ± 0.15	0.13 ± 0.08	0.09 ± 0.03	0.07 ± 0.02
C24:0	4.59 ± 4.99[a]	1.61 ± 0.96[ab]	4.46 ± 0.71[a]	0.91 ± 0.29[b]
Σ SFA	32.63 ± 5.65[a]	31.25 ± 6.12[a]	34.59 ± 5.73[a]	137.22 ± 12.98[b]
C14:1	0.17 ± 0.14	0.28 ± 0.03	0.27 ± 0.04	0.17 ± 0.04
C15:1	0.56 ± 0.34	0.60 ± 0.08	0.64 ± 0.11	0.46 ± 0.17
C16:1	3.96 ± 2.01	3.13 ± 0.54	2.58 ± 0.75	2.83 ± 1.61
C18:1	5.35 ± 4.26[a]	5.78 ± 1.91[a]	4.32 ± 1.74[a]	80.90 ± 16.21[b]
C20:1	1.38 ± 0.93	1.81 ± 0.21	2.17 ± 0.67	1.56 ± 0.40
C22:1	0.08 ± 0.06	0.10 ± 0.09	0.12 ± 0.08	0.11 ± 0.05
C24:1n-9	0.40 ± 0.35	0.54 ± 0.39	0.60 ± 0.23	0.22 ± 0.10
Σ MUFA	11.90 ± 3.97[a]	12.05 ± 2.97[a]	10.71 ± 2.28[a]	86.25 ± 3.92 [b]
C16:2	3.33 ± 3.48	6.06 ± 0.32	5.91 ± 0.39	2.62 ± 0.39
C16:3	0.55 ± 0.70	1.18 ± 0.37	0.89 ± 0.54	0.32 ± 0.07
C16:4	1.04 ± 0.89	2.64 ± 0.96	1.14 ± 0.61	0.26 ± 0.08
C18:2n6	3.00 ± 2.51[a]	4.38 ± 1.27[a]	3.33 ± 1.36[a]	53.73 ± 11.46[b]
C18:3n6	0.42 ± 0.24	0.31 ± 0.06	0.33 ± 0.10	1.04 ± 0.38
C18:3n3	2.52 ± 1.69	1.90 ± 0.37	2.21 ± 0.43	5.45 ± 1.19
C18:4n3	1.74 ± 1.50	0.96 ± 0.25	0.84 ± 0.06	0.49 ± 0.24
C20:2n6	1.37 ± 1.38	1.50 ± 0.23	1.77 ± 0.52	0.65 ± 0.24
C20:3n6	0.51 ± 0.43	0.74 ± 0.09	1.00 ± 0.41	0.34 ± 0.07
C20:4n6	1.71 ± 1.51	3.01 ± 0.46	3.44 ± 1.55	0.81 ± 0.48
C20:3n3	0.20 ± 0.06	0.22 ± 0.05	0.15 ± 0.06	0.10 ± 0.15
C20:4n3	0.37 ± 0.19	0.56 ± 0.11	0.54 ± 0.08	0.20 ± 0.10
C20:5n3	2.18 ± 1.19[a]	2.06 ± 0.43[a]	2.18 ± 0.49[a]	1.46 ± 0.24[a]
C22:2i/2j	2.84 ± 2.41	4.78 ± 0.80	5.20 ± 2.33	1.71 ± 0.98
C21:5	0.88 ± 0.69	0.89 ± 0.40	1.34 ± 0.53	0.55 ± 0.21
C22:5n6	1.57 ± 2.09	1.68 ± 0.41	1.89 ± 0.54	1.15 ± 0.43
C22:5n3	3.56 ± 2.86	2.11 ± 0.79	2.74 ± 123	1.79 ± 0.34
C22:6n3	5.84 ± 3.01[a]	5.94 ± 1.11[a]	6.62 ± 0.39[a]	1.71 ± 0.19[b]
Σ PUFA	33.61 ± 5.71[a]	40.93 ± 6.22[a]	41.51 ± 7.09[a]	79.44 ± 4.14[b]
PUFA n-3	16.40 ± 6.52[a]	13.75 ± 2.36[a]	15.27 ± 1.65[a]	10.02 ± 1.58[b]
PUFA n-6	8.57 ± 2.94[a]	11.64 ± 2.29[a]	11.76 ± 2.33[a]	57.71 ± 7.47[b]
n-3/n-6	2.66 ± 0.5[a]	1.19 ± 0.04[b]	1.32 ± 0.23[b]	0.17 ± 0.04[c]
EPA + DHA	8.02 ± 2.2[a]	8 ± 1.54	8.8 ± 0.87	2.3 ± 0.51[b]
AI	1.18 ± 0.22	1.01 ± 0.04	0.94 ± 0.09	1.2 ± 0.08
TI	0.42 ± 0.16[a]	0.5 ± 0.02	0.47 ± 0.07	1.11 ± 0.14 [b]

Values are mean ± SD, n = 10

Different letters indicate significant differences at $p < 0.05$ between different cooking processes

Principal component analysis (PCA)

PCA was performed including, raw and cooked Ark shells, in order to determine changes that may occur on the biochemical parameters of Ark shells after cooking (Fig. 2). The projection in PC1 allowed us to separate raw individuals from the cooked ones (PC1 76.90%). The variables that contributed the most to the first component

Table 4 Triglycerides profile of the raw (RA) and pan-fried (PF) Ark shells

Fatty acids (mg/g DW)	RA	PF
C14:0	6.98 ± 2.97	5.15 ± 2.54
C15:0	0.21 ± 0.08	0.23 ± 0.09
C16:0	20.79 ± 7.33[a]	48.68 ± 1.40[b]
C17:0	0.22 ± 0.04	0.69 ± 0.08[b]
C18:0	3.11 ± 0.92[a]	0.10 ± 0.00[b]
C20:0	0.58 ± 0.14	0.52 ± 0.13
C22:0	0.15 ± 0.05	0.18 ± 0.07
C24:0	0.16 ± 0.06	0.19 ± 0.10
Σ SFA	25.05 ± 10.83[a]	55.37 ± 0.84[b]
C14:1	0.06 ± 0.02	0.09 ± 0.00
C15:1	0.02 ± 0.01[a]	0.05 ± 0.00[b]
C16:1	1.01 ± 0.20[a]	4.92 ± 1.36[b]
C18:1	25.96 ± 5.11[a]	131.97 ± 20.33[b]
C20:1	0.19 ± 0.08[a]	1.02 ± 0.02[b]
C22:1	0.06 ± 0.00	0.06 ± 0.00
C24:1n-9	0.10 ± 0.00[a]	0.12 ± 0.00[b]
ΣMUFA	27.03 ± 5.59[a]	138.27 ± 18.93[b]
C16:2	0.09 ± 0.02[a]	0.40 ± 0.16[b]
C16:3	0.17 ± 0.09[a]	0.46 ± 0.07[b]
C16:4	0.07 ± 0.00[a]	0.23 ± 0.07[b]
C18:2n6	17.10 ± 9.79[a]	30.80 ± 4.51[b]
C18:3n6	0.30 ± 0.06[a]	0.03 ± 0.00[b]
C18:3n3	2.53 ± 0.75	3.03 ± 0.46
C18:4n3	1.18 ± 0.40	1.60 ± 0.53
C20:2n6	0.04 ± 0.06	0.01 ± 0.00
C20:3n6	0.14 ± 0.08	0.13 ± 0.01
C20:4n6	0.07 ± 0.01[a]	0.10 ± 0.00[b]
C20:3n3	0.10 ± 0.05[a]	0.20 ± 0.01[b]
C20:4n3	0.16 ± 0.04[a]	0.23 ± 0.00[b]
C20:5n3	0.88 ± 0.01[a]	0.66 ± 0.26[b]
C22:2i/2j	0.13 ± 0.01[a]	0.35 ± 0.02[b]
C21:5	0.03 ± 0.00[a]	0.18 ± 0.06[b]
C22:5n6	0.11 ± 0.01	0.13 ± 0.0
C22:5n3	0.19 ± 0.12	0.04 ± 0.01
C22:6n3	1.34 ± 0.59	2.34 ± 0.49
ΣPUFA	23.26 ± 9.79[a]	39.69 ± 4.60[b]
PUFA n-3	5.12 ± 1.23[a]	6.53 ± 0.62[b]
PUFA n-6	17.72 ± 9.73[a]	31.22 ± 4.50[b]

Values are mean ± SD, n = 10

Different letters indicate significant differences ($p < 0.05$) between raw (RA) and pan-fried (PF) Ark shells

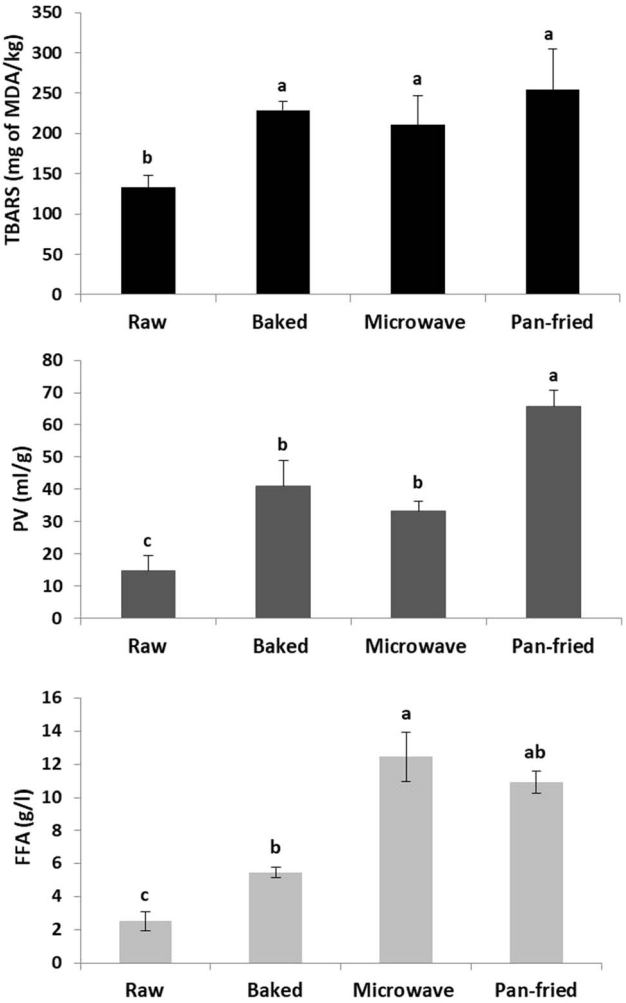

Fig. 1 Lipid peroxidation indices (TBARS, PV and FFA) of raw and cooked Ark shell

were protein, moisture, PUFA n-3, EPA, DHA, n-3/n-6, EPA + DHA and which displayed a clear correlation with raw samples. Indeed, BA and MW samples shared with RA Ark shells high nutritional characteristics. Nevertheless, PF Ark shells were separated from the other groups (PC2 20.90%; Fig. 2). This group was characterized by high amounts of TL, TBARS, PV, FFA, PUFA n-6, SFA, MUFA and TI.

Discussion

Proximate composition of raw and cooked Ark shell

All the domestic cooking treatments used in this investigation considerably affected the proximate composition of Ark shells. Due to heat-provoked evaporation, the moisture content in Ark shells tissues was reduced in all cooking methods. In line with this, the moisture reduction after all cooking treatments may be related to the applied temperature that could denature protein structure by affecting its ability to bond with water (Bejaoui et al. 2019a). Similar

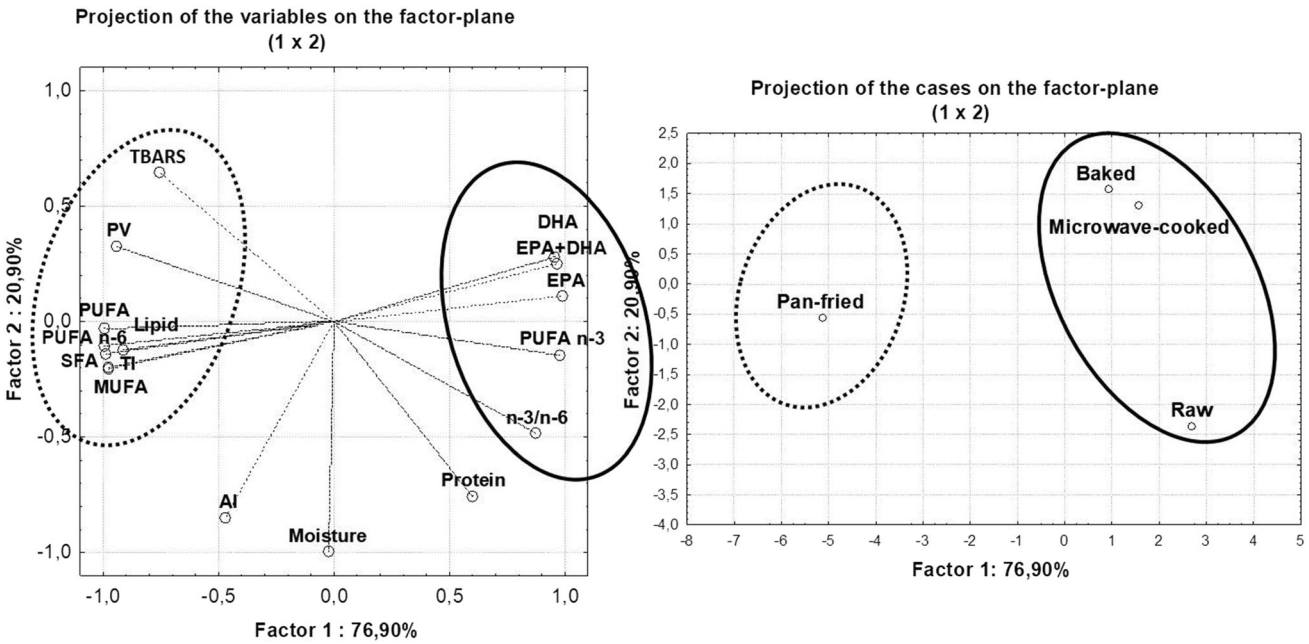

Fig. 2 Principal component analysis (PCA) of raw and cooked Ark shell biochemical parameters and lipid damage indices: **a** projection of the variables on the factor-plane (1 × 2); **b** Projection of the cases on the factor plane (1 × 2)

reports have been demonstrated previously for cooked fish (Kalogeropoulos et al. 2007; Weber et al. 2008). The moisture reduction has been also mentioned as the change that makes lipids rise significantly in cooked fish and bivalves (Ghribi et al. 2017). Our results also corroborate with these findings, showing a significant enhancement of lipid content for MW and PF Ark shells. This increase in fat is probably due to moisture reduction. However, the highest lipid content mentioned in PF Ark shells could also be related to the margarine absorption during the pan-frying treatment after the partial water loss due to evaporation. Hosseini et al. (2014) showed that cooking is responsible for increasing the total fat content of fish, this being more pronounced in fried fish flesh. Besides, the protein content was reduced during all cooking processes ($p < 0.05$). We suppose that, during cooking, the destruction of the muscular part of the Ark shells due to the denaturation process of myofibrillar proteins by heat causes water loss. Similar observations were reported for pork meat by Huang et al. (2011), fish and shrimp by Musaiger and D'Souza (2008), revealing that water loss is linked to protein denaturation as water is mostly kept between the meat muscles structures and cells. Also, Asghari et al. (2013) have demonstrated that the protein was reduced when rainbow trout fillets were cooked by deep-fat frying, indicating extensive absorption of the frying oil.

Fatty acids and nutritional quality indices in raw and cooked Ark shell

Previous studies have shown that FA composition of fresh *A. noae* is closely related to the diet of this species in the Adriatic and Mediterranean Sea (Ezgeta-Balić et al. 2012; Dupčić Radić et al. 2014; Ghribi et al. 2018). Nevertheless, the most changes in the FA profile of cooked Ark shells were observed after pan-frying treatment. For instance, SFA and MUFA contents and their dominated FA such as C14:0, C16:0, C18:0 and C18:1 were higher in PF regardless of RA Ark shells. The most uptake was registered for C18:1. Following this, it has been previously discussed that this FA has higher adhesion and viscosity on the material to be fried, which makes it easily absorbed as compared to other unsaturated FA (Kalogeropoulos et al. 2007). Seafood contained lean flesh that facilitates the absorption of lipids as compared to the over fatty flesh. This hypothesis has been supported by other works carried on pan-fried fish flesh (Weber et al. 2008). Bilgin et al. (2010) reported the richness of margarine butter with SFA and MUFA. These findings were similar to what was signaled in our margarine oil FA analysis (Table 1). However, the significant increase of SFA and MUFA in Ark shells flesh after pan-frying treatment with margarine could probably due to their infiltration from the margarine oil to the fried flesh. In fact, the FA profile of margarine oil before and after cooking confirmed our findings (Table 1).

Before cooking, it was noted that the margarine FA profile was highly rich in oleic acid (C18:1) and linoleic acid (C18:2n6). Meanwhile after pan-frying, these FA were significantly reduced in margarine to be consequently highly accumulated in PF Ark shells flesh, which could confirm the exchange of lipids between the flesh and the frying margarine Sioen et al. (2006). have reported previously the increase of SFA and MUFA contents in codfish after frying with margarine. An even higher increase of SFA and MUFA were, also, found during pan-frying catfish flesh with canola oil (Weber et al. 2008). The important changes of margarine FA composition observed after frying (Table 1) could probably be the cause of PUFA variability in pan-frying treatment. In fact, the highest decreases of PUFA n-3 proportions (e.g. EPA and DHA) reported in PF Ark shell flesh could be explained by the absence of these fatty acids from the margarine oil. Such results have been reported by Orsavova et al. (2015) for corn, sunflower, peanut and canola oils; showing the nonexistence of DHA and EPA in their FA profiles. The significant decrease of the main PUFA n-3 proportions in PF Ark shells are in agreement with several studies carried on fried fish with various oils (Larsen et al. 2010; Zotos et al. 2016). This reduction could be related either to the heat temperature, the frying time or to the reduction of DHA since this FA is known to be sensitive to oxidation during heating treatment. However, as shown in FA composition of frying margarine, C18:2n-6 was the major FA, indicating that the dilution effect caused by extensive oil absorption was responsible for the changes in FA composition in Ark shells flesh upon frying. The previous results confirmed the significant increase of PUFA n-6 content in PF Ark shells. Our findings corroborated with previous studies on PUFA n-3 and PUFA n-6 variation after frying seafood (Hosseini et al. 2014; Ghribi et al. 2017). Concerning the FA composition of BA and MW Ark shell, no remarkable difference was observed when compared to RA.

The alteration of FA compounds could be associated with the loss of nutritional value of flesh and might pose adverse health effects (Li et al. 2011; Bejaoui et al. 2019a). Changes in the nutritional quality of cooked Ark shell flesh were observed only after pan-frying process. The n-3/n-6 is the basic index of equilibrated eicosanoid synthesis in organisms that prevents various health problems (Kinsella et al. 1990). This index was, also, reported as a specific nutritional characteristic of seafood (Bejaoui et al. 2019a). Despite the significant reduction of n-3/n-6 ratio in PF Ark shell flesh, it was within the recommended value (0.25) set by the American Heart Association (Krauss et al. 2000). The current results are in agreement with earlier reports, showing a decrease of n-3/n-6 ratio after frying fish using various vegetable oils (Kalogeropoulos et al. 2007; Ghribi et al. 2017). The long-chain, PUFA n-3 such as EPA (20:5n3) and DHA (22:6n3), are the major FA closely related to lower cardiovascular risk and playing a key role in the biological metabolism of the organism (Hosseini et al. 2014). The EPA + DHA sum, reported in the present study, decreased 4 times in pan-fried Ark shells flesh as compared to the raw one. According to previous nutritional reports, EPA + DHA sum in fried seafood is reduced considerably and approves the influence of this type of cooking on the nutritional quality of seafood (Ghribi et al. 2017). The atherogenicity (AI) and thrombogenicity (TI) indices must be strictly maintained at a low level as they reveal all risks associated with cardiovascular disease (Krauss et al. 2000). For AI results, this index did not vary between fresh and cooked individuals. However, TI increased after the frying process as compared to the raw. Ghribi et al. (2017) reported the same results when seafood was fried with vegetable oils. Both indices remained low even after baking and microwave cooking revealing a good nutritional quality of Ark shells submitted to these cooking treatments. Therefore, eating seafood (e.g. Ark shells) with low AI and TI index may reduce coronary human health risks.

Triacylglycerol (TAG) composition of raw and pan-fried Ark shell

It is well known that exposure to high temperatures and the presence of air alters the unsaturated FA and provoke their oxidation. This process can modify triaglycerol (TAG) structure because at least one of its three FA acyl chains will be modified (Gonzalez-Munoz et al. 1998). The present study revealed a strong increase in the main TAG FA proportions after pan-frying treatment as compared to the raw flesh. We suppose that the hydrolysis of TAG occurred during the frying process, which is considered non-adequat for human health since it increases the risk of several cardiovascular, digestive or other diseases such as cancer (Cox and García-Palmieri 1990). A part from the diversity of vegetable oils, FA and TAG contents are dependent on temperature and heating time. Indeed, some chemical and physical reactions could happen between RA Ark shells flesh and the frying oil that explain chemical changes that happened in fried flesh.

Lipid peroxidation in cooked Ark shell

Following this aspect, the status of lipid peroxidation was evaluated in our present study, considered as one of the major factors limiting the quality and acceptability of seafood products (Kamal-Eldin 2006). The lipid peroxidation was revealed by considerable increases in the levels of TBARS, PV and FFA after all cooking treatments. The

most increase was recorded in PF Ark shells since the used margarine was very rich in SFA which has therefore enhanced the oxidative reaction. This process was previously described for cooked seafood, leading to discoloration, drip loss, development of off-odor and off-flavor, and disruption of cell membranes (Chiou and Kalogeropoulos 2017). It seems that high cooking temperature increase the oxidation processes in Ark shells flesh. Our results are in concordance with other findings showing the harmful effect of pan-frying with vegetable oils, which leads to the enhancement of oxidative alteration (Bejaoui et al. 2019a).

The nutritional quality of seafood during frying will be influenced by the type of oil used by the consumer. Since each oil has a different nature and chemical characteristics, it will have a very specific impact on the cooked tissue (Ghribi et al. 2017). To sum up, the application of PCA analysis confirmed our results showing that RA, BA and MW Ark shells shared the same benefic nutritional characteristics. They formed a single group sharing high n-3 PUFA (e.g. EPA and DHA) and NQI levels, while PF Ark shells showed lower nutritional value as they were characterized predominately by high PUFA n-6 and lipid oxidation products levels.

Conclusion

Our results confirmed that Ark shells nutritional quality changed after cooking. Different cooking treatments (BA, MW and PF) used during this investigation affected the proximate composition, the FA and TAG composition of Ark shells flesh which was more accentuated after pan-frying. During this process, n-3 PUFA (omega 3), n-3/n-6 ratio and EPA + DHA decreased significantly, while n-6 PUFA (omega 6) increased revealing the alteration of Ark shells nutritional value. Lipid oxidation was also observed, during pan-frying, through high TBARS, PV and FFA levels reflecting the impact of this cooking process on this species nutritional traits. Local and foreign consumers are advised when cooking mollusks, particularly Ark shells, to be more attentive about the advantages and risks of each culinary method. Overall, the cooking methods that would optimize the consumption of PUFA n-3 (omega 3) are baking and microwave cooking despite the slight changes observed. It can be concluded that these two methods are suitable for Ark shells cooking. Our choice for heat exposure temperature and cooking time did not adversely compromise the quality characteristics of the baked and microwave cooked Ark shells.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

AOAC, Horwitz W, George W, Latimer JR (eds) (2005) Official methods of analysis of AOAC International, 18th edn. AOAC International. Gaithersburg, MD. U.S.A Official Methods, 2005.08

AOCS, Firestone D (1989) Official methods and recommended practices of the American Oil Chemists' Society, 4th edn. American Oil Chemists' Society, Champaign. Ca 5a-40.

Asghari L, Zeynali F, Sahari MA (2013) Effects of boiling, deep-frying, and microwave treatment on the proximate composition of rainbow trout fillets: changes in fatty acids, total protein, and minerals. J Appl Ichthyol 29:847–853

Bejaoui S, Rabeh I, Ghribi F, Aouini F, Telahigue K, Chetoui I, El Cafsi M (2019) Change in fatty acid composition and evaluation of lipids and protein oxidation in the commercial cooked clams (*Ruditapes decussatus*). Grasas Aceites 70:4

Bejaoui S, Bouaziz M, Ghribi F, Chetoui I, El Cafsi M (2019) Assessment of the biochemical and nutritional values of *Venerupis decussata* from Tunisian lagoons submitted to different anthropogenic ranks. Environ SciPollut Res. https://doi.org/10.1007/s11356-019-06851-y

Bilgin Ş, Bilgin Ş, Zci L, Günlü A, Günlü A, Bolat Y, Bolat Y (2010) Effects of pan frying with different oils on some of the chemical components, quality parameters and cholesterol levels of Rainbow trout (*Oncorhynchus mykiss*). Afr J Biotech 9:6573–6577

Cecchi G, Basini S, Castano C (1985) Méthanolyse rapide des huiles en solvant. Rev Française Corps. Gra n°4.

Chiou A, Kalogeropoulos N (2017) Virgin olive oil as frying oil. Com Rev Food Sci Food Saf 16:632–646. https://doi.org/10.1111/1541-4337.12268

Cox RA, García-Palmieri MR (1990) Cholesterol, triglycerides, and associated lipoproteins. In: Walker HK, Hall WD, Hurst JW (eds) Clinical Methods: The History, Physical, and Laboratory Examinations, 3rd edn. Butterworths: Boston, MA, USA

Dupčić Radić I, Carić M, Najdek M, Jasprica N, Bolotin J, Peharda M, Bratoš Cetinić A (2014) Biochemical and Fatty Acid Composition of Arca noae (Bivalvia: Arcidae) from the Mali Stone Bay, Adriatic Sea. Mediterr Mar Sci 15:520–531. https://doi.org/10.12681/mms.436

Ezgeta-Balić D, Najdek M, Peharda M, Blažina M (2012) Seasonal fatty acid profile analysis to trace origin of food sources of four commercially important bivalves. Aquaculture 334:89–100. https://doi.org/10.1016/j.aquaculture.2011.12.041

Felici A, Vittori S, Meligrana MCT, Roncarati A (2020) Quality traits of raw and cooked cupped oysters. Europ Food Res Tech 246:349–353. https://doi.org/10.1007/s00217-019-03348-3

Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. J BiolChem 226:497–509

Ghribi F, Boussoufa D, Aouini F, Bejaoui S, Chetoui I, Rabeh I, El Cafsi M (2018) Seasonal variation of biochemical composition of Noah's ark shells (Arcanoae L. 1758) in a Tunisian coastal lagoon in relation to its reproductive cycle and environmental conditions. AquatLiv Res 31:14. https://doi.org/10.1051/alr/2018002

Ghribi F, Bejaoui S, Rabeh I, Aouini F, Chetoui I, El Cafsi M (2017) Effects of culinary methods on nutritional characteristics of the edible shellfish Noah's Ark (*Arca noae* L., 1758) from Tunisian coasts. J Aquat Food Prod Tech 26:1324–1336. https://doi.org/10.1080/10498850.2017.1390714

Gonzalez-Munoz MJ, Bastida S, Sanchez-Muniz FJ (1998) Short term in vivo digestibility of triglyceride polymers, dimers, and monomers of thermoxidized palm olein used in deep-frying. J Agric Food Chem 46:5188–5193. https://doi.org/10.1021/jf980598i

Hosseini H, Mahmoudzadeh M, Rezaei M, Mahmoudzadeh L, Khaksar R, Khosroshahi NK, Babakhani A (2014) Effect of different cooking methods on minerals, vitamins and nutritional quality indices of Kutum roach (*Rutilus frisii kutum*). Food Chem 148:86–91. https://doi.org/10.1016/j.foodchem.2013.10.012

Huang F, Huang M, Xu X, Zhou G (2011) Influence of heat on protein degradation, ultrastructure and eating quality indicators of pork. J Sci Food Agric 91:443–448. https://doi.org/10.1002/jsfa.4204

Kalogeropoulos N, Chiou A, Mylona A, Maria S, Nikolaos I, Andrikopoulos K (2007) Recovery and distribution of natural antioxidants (α-tocopherol, polyphenols and terpenic acids) after pan-frying of Mediterranean fin fish in virgin olive oil. Food Chem 100:509–517. https://doi.org/10.1016/j.foodchem.2005.09.072

Kamal-Eldin A (2006) Effect of fatty acids and tocopherols on the oxidative stability of vegetable oils. Eur J Lipid Sci Technol 108:1051–1061. https://doi.org/10.1002/ejlt.200600090

Kinsella JE, Shane K, Kenneth B, Broughton S, Whelan J, Whelan J (1990) Dietary unsaturated fatty acids: interactions and possible needs in relation to eicosanoid synthesis. J Nutr Biochem 1:123–141

Kozul V, Clavic N, Bolotin J, Antolovic N (2011) The experimental rearing of Noah's Ark, Arca noae (Linnaeus, 1758) and Beraded Horse Mussel, Modiolus barbatus (Linnaeus, 1758) in Mali Ston Bay. In: Pospil M (ed) Proceedings of the 46th Croatian and 6th International Symposium on Agriculture and Beekeeping, 2011, February 13–17, Opatija. Zagreb: University of Zagreb, Faculty of Agriculture, Zagreb, Croatia pp 807–809

Krauss RM, Eckel HR, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW, Kris-Etherton P, Goldberg IJ, Kotchen TA et al (2000) AHA dietary guidelines. Revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 102:2284–2299

Larsen D, Young S, Eyres QL (2010) Effect of cooking method on the fatty acid profile of New Zealand King Salmon (*Oncorhynchus tshawytscha*). Food Chem 119:785–790. https://doi.org/10.1016/j.foodchem.2009.07.037

Li Q, Yang L, Ke Q, Kong L (2011) Gametogenic cycle and biochemical composition of the clam Mactra chinensis (Mollusca: Bivalvia): implications for aquaculture and wild stock management. Mar Biol Res 7:407–415

Lowry OH, Rosebrough NJ, Farr AL, Randal RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Marques A, Teixeira B, Barrento S, Anacleto P, Carvalho ML, Nunes ML (2010) Chemical composition of atlantic spider crab *Maja brachydactyla*: human health implication. J Food Compos Anal 23:230–237. https://doi.org/10.1016/j.jfca.2009.10.007

Masson E, Debucquet G, Fischler C, Merdji M (2016) French consumers' perceptions of nutrition and health claims: a psychosocial-anthropological approach. Appetite 105:618–629

Musaiger AO, D'Souza R (2008) The effects of different methods of cooking on proximate, mineral and heavy metal composition of fish and shrimps consumed in the Arabian gulf. Archiv Latin americanos de Nutrición 58:103–109

Oehlenschlager J (2012) Seafood: nutritional benefits and risk aspects. Int J VitamNutr Res 82:168–172. https://doi.org/10.1024/0300-9831/a000108

Olsen RE, Henderson RJ (1989) The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry. J Exp Mar Biol Ecol 129:189–197. https://doi.org/10.1016/0022-0981(89)90056-7

Orsavova J, Misurcova L, VavraAmbrozova J, Vicha R, Mlcek J (2015) Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. Int J MolSci 16:12871–12890. https://doi.org/10.3390/ijms160612871

Paspatis M, Maragkoudaki D (2005) Bivalve fisheries in the gulf of Kalloni, Lesvos island. Present status. In: Proceedings of the 12th Panellenic Ichtyhologists Conference

Peharda M, Ezgeta-Balic D, Davenport J, Vrgoc N (2013) The potential for aquaculture of the bearded horse mussel (Modiolus barbatus) and Noah's Ark shell (Arca noae) in southern Croatia. Aquac Int 21:639–653

Sioen I, Haak L, Raes K, Hermans C, De Henauw S, De Smet S, Van Campa J (2006) Effects of pan-frying in margarine and olive oil on the fatty acid composition of cod and salmon. Food Chem 98:609–617. https://doi.org/10.1016/j.foodchem.2005.06.026

Turan H, Sonmez G, Kaya Y (2007) Fatty acid profile and proximate composition of the thornback ray (*Raja clavata*; L, 1758) from the Simon coast in the black sea. J Fish Sci 1:97–103. https://doi.org/10.3153/jfscom.2007012

Ulbricht TLV, Southgate DAT (1991) Coronary heart disease: seven dietary factors. Lancet 338:985–992

Unusan N (2007) Change in proximate amino acid and fatty acid contents in muscle tissue of rainbow trout (*Oncorhychus mykiss*) after cooking. Int J Food Sci Technol 42:1087–1093. https://doi.org/10.1111/j.1365-2621.2006.01354.x

Weber J, Bochi VC, Ribeiro CP, Victorio AM, Emanuelli E (2008) Effect of different cooking methods on the oxidation, proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. Food Chem 1:140–146. https://doi.org/10.1016/j.foodchem.2007.05.052

Zotos A, Gotsis D, Tsolakis M (2016) The effect of prior frozen storage (−20 °C) on the quality of oven-baked sardines (*Sardina pilchardus*). J Aquat Food Prod Technol 25:240–250. https://doi.org/10.1080/10498850.2013.844217

Župan I, Peharda M, Ezgeta-Balić D, Šarić T (2012) Noah's ark shell (*Arca noae* Linnaeus, 1758): what do we need to know for starting up its aquaculture? Croat J Fish 70:71–81

Župan I, Peharda M, Dolenec T, Dolenec M, Žvab Rožič P, Lojen S, Ezgeta-Balič D, Arapov J (2014) Aquaculture assessment of Noah's Ark (Arca noae Linnaeus, 1758) in the Central Adriatic Sea (Croatia). J Shellfish Res 33:433–441