

PHOSPHOLIPIDS PROFILE OF THE EDIBLE CLAMS FLESH DURING DIFFERENT FRYING PROCESSES

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ملخص

دراسة عن تركيبة الفوسفوليبيد في أنسجة المحار الصالحة للأكل أثناء عمليات مختلفة من القلي: تم تقييم آثار معالجة القلي، باستخدام زيوت مختلفة (زيت الذرة وزيت الزيتون البكر الممتاز وزيت السمك) على تركيبة الفسفوليبيد (PL) ومؤشرات الجودة الغذائية والكيميائية على معظم المحار التجارية في تونس. أسفرت معالجة القلي عن تغييرات كبيرة على الزيوت المشبعة (SFA)، غير المشبعة (MUFA) وغير المشبعة المتعددة (PUFA) في الزيوت و المحار المقلي ($P < 0.05$). في جميع الحالات، تسببت طريقة القلي في تدهور مهم للمركبات الرئيسية للفوسفوليبيد، وترجمتها الزيادات في مستويات C16: 0، C18: 0، C16: 1، C18: 1، n-6، C18: 1، الأراكيدونيك (ARA) وأحماض اللينوليك (C18: 2n-6)، في حين لوحظ انخفاض في مستويات n-3 و docosaheptaenoic (DHA) و ecosapentaenoic (EPA) بشكل رئيسي في الأنسجة المارجرين المقالية، يليها زيت الذرة. كشفت نتائجنا انخفاض في مؤشرات الجودة الغذائية في جميع المحار المقالية ($P < 0.01$). بالإضافة إلى ذلك، تم تقييم مؤشرات الصف الكيميائي باستخدام حمض الثيوباربيتوريك (TBAR)، والبيروكسيد (PV) والأحماض الدهنية الحرة (FFA)، مما أدى إلى زيادة كبيرة في جميع المحار المقالية.

الكلمات المفتاح: القلي، تكوين الفوسفوليبيد، مؤشرات الصف الكيميائي، الزيوت

RESUME

Profil de phospholipide des tissus comestibles de palourde au cours de différents processus de friture :

Les effets du traitement par la friture, utilisant diverses huiles gastronomiques (huile de maïs, huile d'olive extra vierge et huile de margarine) sur la composition des phospholipides (PL), des indices de la qualité nutritionnelle et chimique ont été évalués sur les palourdes les plus commerciales (*Venerupis decussata*) en Tunisie. Le traitement de la friture a entraîné des modifications significatives des acides gras saturés (AGS), monoinsaturés (AGMI) et polyinsaturés (AGPI) des tissus frits et des huiles frites ($p < 0,05$). Dans tous les cas, la méthode de friture a provoqué une importante modification des principaux composés de la fraction phospholipidique traduite par des augmentations des taux de C16: 0, C18: 0, C16: 1, C18: 1, n-6 AGPI, arachidonique (AAR) et linoléique (C18: 2n- 6) acides, alors que des réductions de taux de n-3 AGPI, docosaheptaénoïques (ADH) et écosapentaénoïques (AEP) ont été observées principalement dans les tissus frits avec la margarine, suivis par l'huile de maïs.

Nos résultats ont révélé une réduction des indices de la qualité nutritionnelle (n-3/n-6 AGPI, EPA + DHA et DHA / C16: 0) chez toutes les palourdes frites ($p < 0,01$). En outre, les indices de qualité chimique ont été évalués à l'aide de l'acide thiobarbiturique (TBAR), de l'indice de peroxyde (PV) et des acides gras libres (FFA), ce qui a entraîné une élévation significative de ces indices chez toutes les palourdes frites. Prenant toutes les données ensemble, les tests de friture avec l'huile de margarine ont induit une altération significative de la composition en phospholipides. Toutefois, la friture avec l'huile d'olive semble être la solution la plus saine pour la nutrition et la santé des humains.

Mots-clés: Friture, composition en phospholipides, indices de qualité chimique, huiles

ABSTRACT

The effects of frying treatment using diverse gastronomic oils (corn oil, extra virgin olive oil and margarine oil) on the composition of the phospholipids (PLs), nutritional and chemical quality indices were evaluated on the most commercial clams (*Venerupis decussata*) in Tunisia. The frying treatment resulted in significant changes on saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in almost fried tissues and fried oils ($p < 0.05$). In all cases, frying method caused important enhancement of the main phospholipids compounds as evidence by the increasement of C16:0, C18:0, C16:1, C18:1, n-6PUFA, arachidonic (ARA) and linoleic (C18:2n-6) acids, while reductions of n-3 PUFA, docosaheptaenoic (DHA) and ecosapentaenoic (EPA) acids were observed mostly in the fried tissues with margarine followed by corn oil. Our results revealed depletions of the nutritional quality indices (n-3/n-6 PUFA, EPA+DHA and DHA/C16:0) in all fried clams ($p < 0.01$). Besides, the chemical quality indices were assessed through thiobarbituric acid (TBAR), peroxide value (PV) and free fatty acids (FFA), resulting in a significant elevation of these indices in all fried clams. Taken all the data together, frying tests with margarine oil induced a significant enhancement of phospholipids composition. However, frying with olive oil seems to be safer for the nutrition and health of humans.

Keywords: Frying, clams, phospholipids composition, chemical quality indices, oils.

INTRODUCTION

Lipids play a crucial role in human nutrition that comprises growth, therapeutic prevention of diseases, development of human embryo, and prevention against many serious diseases (cardiovascular, inflammation etc) (Kinsella et al., 1990), while the main role of lipids in human health is succeeding since more investigation is being done. Bivalves, such as clam is considered as a healthy food because it contain an important amount of biochemical composition as well as phospholipids, that enclose omega-3 fatty acids (n-3 PUFA) (Liu et al., 2019). Phospholipids (PLs) are known as one of the chemical foundations of life since; they include the major membranes component and contribute as flavor precursors in the nutrition method (Malavolta et al., 2004). These components are produced from a combination of molecules (glycerol, phosphorylated alcohol, fatty acid) (Wang et al., 2010b). Due to their molecular arrangement and the high unsaturated fatty acid content, phospholipids have an important potential to hydrolysis and therefore of oxidation (Ademowo et al., 2017; Reis and Spickett, 2012).

In this regards, cooking process with vegetable oils have been reported to induce harmful effect in the fatty acid composition of meat (Şişik Oğraş et al., 2018), pork (Amici et al., 2015), chicken (Bolger et al., 2017), and seafood (Ghribi et al., 2017; Bejaoui et al., 2019). While few data were demonstrated that cooking with oils has an effective effect on the phospholipids composition of various types of tissues (Wang et al., 2010b; Liu et al., 2019).

Frying is a technique processing with rapid heat transfer and difficult exchanges between oil and the fried food and being commonly used in the processing in seafood mainly bivalves (Wright et al., 2018). This process is generally used in the restoration, the hotels and also it is very appreciated at the international level mainly in the coastal countries such as Cadiz in the south of Spain (FWS, 2017). Frying process can widely change the taste, texture, and nutritional qualities of bivalves resulting by flavor, and color production as well as protein degeneration, lipid oxidation, (Otles and Sengor, 2005; Ghribi et al., 2017). Lipid exchange and the oxidation of the fried bivalves, may impact the particular nutritional characteristic related to its lipid components (Lui et al., 2019). However, to the best of our knowledge, little information about the impact of frying process on the lipids in shellfish, especially clam, is available. Few data have demonstrated that phospholipids composition of seafood was affected by culinary treatment (Cui and Decker 2016; Lui et al., 2019). Among them, frying process is known by having a marginal effect on nutrient losses than other cooking processes and considered to improve the nutritive quality by absorbing frying oils into the fried

food which is rich in vitamin E and unsaturated fatty acids (Fillion and Henry, 1998).

In this regards, we aimed to analyze the changes occurred in phospholipids composition in the commercial clam (*Venerupis decussata*) with different frying methods. Also, the oxidation process was evaluated through several indices (peroxide value, free fatty acid and thiobarbituric acid).

MATERIALS AND METHODS

Sampling clams and tissues preparation

Clams were provided by the mutual societies of shellfish farmers of Bizerte (S.M.C.B). The edible flesh were removed and washed before frying process, then, divided into four lots of 30 each as follows: The first one was considered as the control and the other three were fried with corn, olive and margarine oils using deep fryer (MOULINEX AF123111 UNO M, France) related to an electrical heating unit (preheated to 185 ± 5 °C) for 4–5 min.

Lipid classes' separation

Total lipids were extracted with the mixture of chloroform/methanol (2v/1v), containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. Lipid classes were separated using thin-layer chromatography (TLC) with one dimensional double development based on Olsen and Henderson (1989) method. Briefly, 500 µL of lipid extracts of each samples were separated on silica gel plates (20x20 cm, Merck, Germany). To obtain phospholipids fraction, silica plates were fixed in a mixed solvent containing methyl acetate (25V), isopropanol (25V), chloroform/methanol (25: 10V) and potassium chloride (0.25%). When migration is accomplished, silica plates containing lipid classes were spraying with 2'-7' dichloro-fluorescein (0.1%) and observed under UV light after. All visualized fractions were methylated and injected in CPG. Results were expressed as percent (%) of the total fatty acids.

Quality indices

Nutritional quality indices (n-3/n-6; EPA+DHA and DHA/C16:0) were determined based on the method of Marques et al. (2010) and El Reffaei et al. (2014) respectively.

Chemical quality indices: Thiobarbituric acid (TBAR) was determined spectrophotometrically at 532 nm according to the AOCS (1989) method. Peroxide value (PV) was also determined according to the method of AOCS (1989). Free fatty acids (FFA) measurement was analyzed according to AOCS (1989) method.

Statistical analysis

Results were presented in tables and figures as mean \pm standard deviation (SD). The normality of distributions and the homogeneity of variances of the obtained results were performed using Kolmogorov-Smirnov test. Significant differences between fresh

and fried tissues were carried out using Statistica version 8.00 software (Chicago, USA) through Tukey test (ANOVA one-way test).

RESULTS

Phospholipids composition of the fried oils

PLs compositions of the fried oils are presented in Table I. Our results revealed that corn oil was predominated by polyunsaturated fatty acids (PUFA) followed by monounsaturated (MUFA) and saturated (SFA) fatty acids. However, MUFA was the major compound that dominates the composition of olive and margarine oils ensured with SFA and PUFA.

PLs compositions were affected by heating treatments in all fried oils ($p < 0.05$) (Table I). Concerning corn oil, SFA mainly C14:0, C16:0 and C18:0 were decreased significantly after frying process ($p < 0.01$), while PUFA was increased. This enhancement was grouping essentially n-6PUFA such as C18:2n-6 and C18:3n-6 which increased by 26% and 58% respectively when compared with the fresh composition. However, MUFA remained stable after frying process.

After frying, the composition of olive oil was enhanced significantly as evidence by increases of SFA and PUFA levels with 15% and 19% respectively (Table I). Such increase was supported by increases of C14:0, C15:0, C16:0, C18:0 as well as n-3 PUFA mainly C18:2n-6 ($p < 0.01$). Dissimilar variation was recorded for MUFA, C16:1 and C18:1 showing significant decreases by 45%, 44% and 46% respectively in the fried oil than the fresh one.

The PLs compositions of margarine used in the frying process is showed in Table I. Fried margarine was characterized by the highest proportions of SFA (27%), C14:0 (181%), C15:0 (311%) and C16:0 (13%). Similar trends were observed for PUFA, C18:2n-6 and C18:3n-6 which increased significantly in relation to the fresh one ($p < 0.05$). According to our results, there was a tendency towards lower levels of MUFA in the fried margarine which was characterized with a significant decrease of C16:1 and C18:1 ($p < 0.001$).

However, no differences were found for n-3 PUFA in all fried oils as compared to the fresh ones.

Table I: Phospholipids levels in the fried oils before and after treatments.

% of TFA	Corn oil		Olive oil		Margarine oil	
	OBF	OAF	OBF	OAF	OBF	OAF
C14:0	1.80±0.25	1.00±0.65 ^b	1.05±0.21	0.45±0.02 ^c	1.96±0.63	5.51±0.45 ^c
C15:0	0.96±0.34	1.02±0.89	0.33±0.11	0.13±0.04 ^b	0.36±0.22	1.48±0.38 ^c
C15:1	12.16±1.45	11.52±2.31	0.32±0.06	0.33±0.01	8.54±0.52	15.65±1.42 ^c
C16:0	19.85±1.03	15.07±1.84 ^b	20.88±2.69	23.12±1.47 ^a	23.89±2.08	27.19±1.90 ^b
C16:1	4.29±0.30	4.31±0.19	0.89±1.87	0.49±0.06 ^b	0.74±0.02	2.18±0.22 ^c
C16:2	5.53±0.09	5.50±0.60	2.23±0.39	1.64±0.71 ^b	5.83±0.18	9.99±1.02 ^a
C16:3	0.48±0.14	0.42±0.08	0.15±0.06	0.16±0.03	0.14±0.01	0.26±0.03 ^a
C16:4	0.82±0.02	1.04±0.77	0.39±0.07	0.41±0.08	0.33±0.00	1.43±0.22 ^b
C18:0	6.91±0.74	3.72±0.15 ^c	1.48±0.31	3.63±0.59 ^c	3.39±0.39	3.45±0.57
C18:1	17.81±1.46	16.52±1.67	51.32±5.88	27.51±2.91 ^c	39.20±0.92	18.15±1.45 ^c
C18:2n-6	22.25±2.87	28.10±2.01 ^b	15.48±4.05	20.73±0.97 ^b	12.90±1.38	10.17±1.74 ^a
C18:3n-6	4.77±0.11	7.57±0.97 ^a	0.39±0.09	0.57±0.02 ^a	0.64±0.05	1.19±0.07 ^a
C18:3n-3	1.70±0.44	1.41±0.56	4.04±0.12	3.60±0.74	0.66±0.11	0.70±0.21
C18:4n-3	1.80±0.20	2.06±0.22	0.50±0.09	0.46±0.10	1.36±0.55	1.40±0.31
SFA	29.59±2.34	20.89±3.05 ^b	23.75±2.02	27.33±2.07 ^c	29.61±1.42	37.64±2.36 ^b
MUFA	34.75±4.01	32.35±4.10	52.54±5.00	28.54±1.51 ^c	48.49±3.28	35.99±1.85 ^c
PUFA	37.38±2.61	45.10±2.84 ^b	22.80±1.08	27.18±3.11 ^c	21.89±1.11	26.35±3.68 ^a
PUFA n-3	3.51±0.15	3.471±0.45	5.03±0.41	3.96±1.77	2.03±2.85	3.30±0.44
PUFA n-6	27.02±1.08	30.67±0.99 ^a	15.88±0.99	21.30±0.84 ^b	13.54±2.50	11.36±1.87 ^a

Results are expressed as means ± SD (n=6).

Percentages (%) with different superscript letters (a, b, c) are significantly different (ANOVA, Tukey's HSD test)

Means of fresh tissues as compared to frying ones are significantly different at 5% as follows: fresh tissues vs frying tissues $a < 0.05$; $b < 0.01$ and $c < 0.001$.

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; OBF: oil before frying; OAF: oil after frying.

Phospholipids composition of the fried edible flesh Changes in the phospholipids composition of the fried edible flesh with corn oil

The PLs composition of clams flesh after frying process with corn oil is presented in Table II. Fried

flesh contained high levels of SFA (18%), C15:0 (119%), C16:0 (21%) and C18:0 (20%). Our results revealed significant increases of PUFA levels mainly n-6 PUFA which was characterized by a statistical rise of C18:2n-6, C18:3n-6, C20:2n-6, arachidonic

(C20:4n-6) and C22:5n-6 ($p<0.05$). However, n-3 PUFA and its major component eicosapentaenoic (EPA) and docosahexaenoic (DHA) decreased significantly by 33%, 47% and 37% respectively when compared to the fresh tissues ($p<0.05$). In the fried tissues MUFA levels did not vary as compared to the fresh one.

Changes in the phospholipids composition of the fried edible flesh with olive oil

PLs levels after frying with olive oil are shown in Table II. SFA level in fried flesh did not differ significantly from the fresh one. However, MUFA level was significantly increased in the fried flesh reaching highest levels for C15:1 (2.062%), C16:1 (5.609%) and C18:1 (13.642%). Also, PUFA level increased significantly and was approximately 1 time higher than the fresh one. Similarly, n-6 PUFA mainly ARA and its precursor C18:2n-6 increased with 147%, 153% and 436% respectively when

compared to fresh one. The greatest decreases in n-3 PUFA, EPA and DHA were observed in fried clams ($p<0.05$).

Changes in the phospholipids composition of the fried edible flesh with margarine oil

Changes in PLs composition of fried clams with margarine oil are shown in Table II. Comparison to the fresh tissues, significant increases of SFA (48.685%), MUFA (23.791%) mainly C18:0 (10.091%), C16:0 (29.614%), C15:0 (0.863%), C14:0 (7.638%), C16:1 (5.477%) and C18:1 (14.083%) were observed ($p<0.05$). However, PUFA level decreased significantly. This decrease was supported by significant reduction of n-3PUFA especially EPA and DHA as compared to the fresh one. Dissimilar trends were observed for n-6PUFA, C18:2n-6, C20:2n-6, ARA and C22:5n-6 which reveal statistical increases after fried process.

Table II: Phospholipids levels the edible clams' tissues before and after frying processes.

% of TFA	FT	FTC	FTO	FTM
C14:0	5.709 ±0.262	5.367 ±0.394	5.571 ±0.390	7.638 ±0.361 ^b
C15:0	0.456 ±0.000	1.000 ±0.115 ^b	0.552 ±0.043	0.863 ±0.000 ^c
C15:1	1.070 ±0.099	1.294 ±0.213	2.062 ±0.104 ^b	1.171 ±0.099
C16:0	21.636 ±0.764	26.343 ±1.541 ^b	21.576 ±1.758	29.614 ±0.673 ^b
C16:1	4.884 ±0.058	3.982 ±0.831	5.609 ±0.335 ^a	5.477 ±0.415 ^b
C16:2	1.148 ±0.391	2.019 ±0.461	2.682 ±0.685 ^a	1.171 ±0.982
C16:3	2.559 ±0.281	2.359 ±0.724	3.113 ±0.370	2.046 ±0.798
C16:4	1.369 ±0.579	1.092 ±0.711	0.907 ±0.048	1.327 ±0.014
C18:0	8.368 ±0.399	10.046 ±0.615 ^a	8.962 ±0.422	10.091 ±0.062 ^b
C18:1	7.561 ±0.360	8.698 ±0.937	13.642 ±0.879 ^b	14.083 ±1.161 ^b
C18:2n-6	0.776 ±0.031	4.740 ±0.982 ^b	4.161 ±0.533 ^b	5.853 ±0.435 ^b
C18:3n-6	0.565 ±0.001	1.187 ±0.028 ^c	1.220 ±0.036 ^b	0.579 ±0.082
C18:3n-3	1.515 ±0.058	1.720 ±0.521	1.130 ±0.077 ^b	1.193 ±0.002 ^b
C18:4n-3	1.139 ±0.126	1.449 ±0.330	0.772 ±0.056	0.473 ±0.087 ^a
C20:0	0.122 ±0.017	0.144 ±0.002 ^a	0.140 ±0.006	0.170 ±0.029
C20:1	2.226 ±0.254	2.008 ±0.033	1.989 ±0.168	2.056 ±0.402
C20:2n-6	0.075 ±0.005	0.728 ±0.074 ^c	0.735 ±0.059 ^b	1.943 ±0.043 ^c
C20:3n-6	0.325 ±0.024	0.311 ±0.075	0.278 ±0.022	0.219 ±0.052
C20:4n-6	1.383 ±0.040	3.694 ±0.238 ^c	3.502 ±0.273 ^c	3.950 ±0.020 ^c
C20:3n-3	0.195 ±0.023	0.142 ±0.095	0.154 ±0.038	0.180 ±0.015
C20:4n-3	0.358 ±0.037	0.226 ±0.013	0.206 ±0.023 ^a	0.242 ±0.058
C20:5n-3	5.526 ±0.0493	2.904 ±0.397 ^a	3.518 ±0.318 ^a	2.984 ±1.525 ^b
C22:0	0.133 ±0.014	0.123 ±0.015	0.149 ±0.001 ^a	0.149 ±0.012
C22:1	0.678 ±0.036	0.634 ±0.078	0.562 ±0.072	0.903 ±0.357
C22:2n-6	0.802 ±0.079	0.936 ±0.090	1.355 ±0.066 ^b	0.772 ±0.094
C22:5n-6	0.346 ±0.184	1.083 ±0.139 ^a	0.308 ±0.035	0.954 ±0.036 ^a
C22:5n-3	2.030 ±0.322	2.267 ±0.332	2.715 ±0.168	1.660 ±0.253
C22:6n-3	11.882 ±0.685	7.370 ±0.522 ^b	8.506 ±0.975 ^a	1.076 ±0.044 ^b
SFA	37.431 ±1.388	44.327 ±2.360^b	38.843 ±2.103	48.685 ±1.472^c
MUFA	16.420 ±0.808	16.619 ±1.782	24.865 ±0.934^b	23.791 ±0.600^a
PUFA	31.999 ±0.943	34.234 ±2.429^a	34.367 ±1.076	26.629 ±0.920^b
PUFA n-3	22.647 ±0.386	15.081 ±1.254^b	17.103 ±1.149^b	7.864 ±1.388^b
PUFA n-6	4.274 ±0.132	11.682 ±0.680^b	10.560 ±0.288^c	13.973 ±0.297^b

Results are expressed as means ± SD (n=6).

Percentages (%) with different superscript letters (a, b, c) are significantly different (ANOVA, Tukey's HSD test)

Means of fresh tissues as compared to frying ones are significantly different at 5% as follows: fresh tissues vs frying tissues **a**<0.05; **b**<0.01 and **c**<0.001.

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; F: fresh tissues; OBF: oil before frying; OAF: oil after frying; FTC: fried tissue in corn oil; FTO: fried tissue in olive oil; FTM: fried tissue in margarine; ND: No determined

Changes in the nutritional quality of fried clams

Three indices reflecting the nutritional quality of fresh and fried tissues were determined in the present study (Figure 1). Our results revealed a significant reduction in the n-3/n-6, DHA/C16:0 ratios and

EPA+DHA sum in all fried tissues as compared to the fresh one ($p<0.05$). Clams fried with margarine were the most affected one, resulting with great decreases by 89%, 76% and 93% for n-3/n-6, EPA+DHA and DHA/C16:0 respectively.

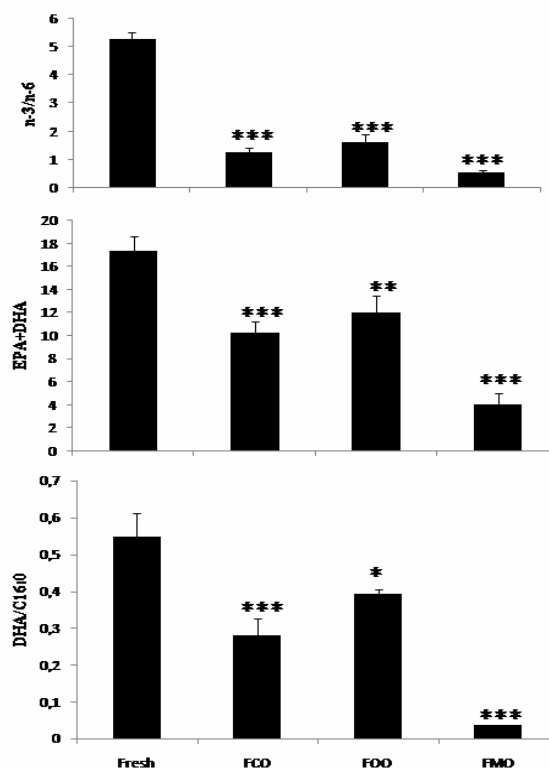


Figure 1: Changes in the nutritional quality of clams after fried process.

Fresh vs Fried tissues are presented with * <0.05 ; ** <0.01 and *** <0.001 (ANOVA, Tukey's HSD test)

FCO: tissues fried with corn oil; FOO: tissues fried with olive oil; FMO: tissues fried with margarine oil.

Chemical quality indices

Changes of the chemical quality indices after frying process are presented in Table III. As indicated in Table III, PV showed significantly increase in all fried tissues. This increasement was higher than the permissible limit (10-20) especially for fried tissues with corn (37.01meq/kg) and margarine (50.62meq/kg) oils.

Significant increase of TBAR level was observed in fried tissues corn and margarine ($p<0.01$). However,

fried tissues with olive oil did not change as compared to fresh one. Although, these values were within the permissible limits (Table III).

Similar trends were observed for FFA, revealing significant enhancement after frying process with corn and margarine oils, while clams fried with olive oil did not change. Our results indicated that FFA level of fried tissues with margarine were higher than the permissible limit of 7g/100g.

Table III: Chemical quality indices in fresh and fried clams' tissues

	Fresh	Corn	Olive	Margarine	Permissible limit
PV	7.00±0.54	37.01±1.25 ^c	18.74±1.07 ^c	50.62±2.09 ^c	10-20 meq/Kg (Connell 1975)
TBAR	2.51±0.09	3.55±0.22 ^b	3.00±0.47	4.00±0.25 ^c	8 mg MDA/kg (Ozden and Erkan, 2006)
FFA	3.22±0.36	7.54±0.66 ^c	3.79±0.44	11.69±1.11 ^c	7 g/100g (IFOMA, 1981)

Results are expressed as means ± SD (n=9).

Percentages (%) with different superscript letters (a, b, c) are significantly different (ANOVA, Tukey's HSD test)

Means of fresh tissues as compared to frying ones are significantly different at 5% as follows: fresh tissues vs frying tissues: a <0.05 ; b <0.01 and c <0.001 .

PV: peroxide value; TBAR: Thiobarbituric acid; FFA: free fatty acids.

DISCUSSION

Researchers have independently indicated that frying process may affect the nutritional quality of seafood mainly the fatty acids (Ansorena et al., 2010; Ghribi et al., 2017). But, few data was available on the effect of frying process on the PLs composition in fish and shellfish tissues (Boselli et al., 2012; Liu et al., 2019). So, we investigate for the first time the effect of three frying oils (corn, olive and margarine) on the composition of PLs in the clams (*Venerupis decussata*) from the Tunisian market. The choice of this species is based on its abundance, its national and international commercialization and also the absence of the scientific works related to this issue.

During our frying process, major changes were observed in the composition of PLs. Increases in the main PLs such SFA and MUFA were occurred mostly in margarine fried tissues. However, PUFA levels decreased significantly, confirming by a significant diminution of n-3 PUFA mainly EPA and DHA. For corn oils fried tissues, there was an increase of SFA and PUFA levels and their essential compounds, while MUFA showed a similar variation. Such increases of SFA could be related to the oil composition which was previously described as rich in SFA mainly C16:0 and C18:0. For PUFA this increase was linked firstly to the elevation of n-6 PUFA levels, ARA and C18:2n-6. In contrast, fried clams with olive oil showed a low effect as compared to the other condition. As compared to the fresh tissues, no significant variations were recorded for SFA and PUFA but their compounds showed a minor variation. Concerning MUFA, a slight increase was reported in the fried tissues with olive oil. Our results could be attributed mostly to oils penetration and fried temperature which influence the metabolism of PLs and provoke membrane destruction through the initiation of lipid peroxidation processes. Likewise, PL is principally the ester membrane, which contains more PUFA (Duckett et al. 1993). Deep frying with corn and olive oils have no observable influence on the PUFA levels of PL fraction in the fried tissues, but the contents of PUFA after pan frying with margarine was practically decreased as compared to the fresh tissues. This phenomenon probably could be explained by the different frying process and/or by the faster penetration of PUFA from tissues (especially n-3) into the fried margarine. Our results were in accordance with Liu et al., (2019) shown a decrease of PLs in *R. philippinarum* after frying with soybean and sunflower oils. Also, Bakar et al. (2008) found that frying had certain effects on the PL levels of fried mackerel fish meat.

Our data showed that frying treatment with corn, olive and margarine oils provoked a significantly decreases of EPA+DHA, n-3/n-6 PUFA and DHA/C16:0 in all fried clams. According to our

results, margarine appeared to be more vulnerable method for the quality of fried clams, while olive had the less effect comparatively with fresh flesh, appears to be the most suitable method for clams frying. This result was in concordance with recent investigation shown that frying in extra virgin olive oil was actually healthier than other process (Echevarría et al., 2016).

Analysis of lipids and nutritional quality of the fried clams showed that the composition of PLs affected significantly. From a dietetic point of view, during the frying process, certain changes took place; especially the processes of fat oxidation and hydrolysis. As shown in our study, many investigations have reported that oxidation of lipids can reduce the nutritional value and safety of seafood by the development of secondary products such as Thiobarbituric acid (TBAR) which are known to be dangerous for health since they are related with membrane damage, heart disease, depression and cancer growth (Suja et al. 2004). In the present study, TBAR, PV and FFA were calculated to evaluate the susceptibility of PLs to oxidation. Our study showed that clams tissues were more vulnerable to oxidation during frying with vegetable oils suggests that pan frying with margarine provokes a higher thermal-oxidation than deep frying with corn and olive oils. Also our results revealed that olive oil is safer than corn oil for heating at frying temperatures. Our data were in harmony with other research reported that a high value of TBAR, PV and FFA in fried seafood, indicates generation of lipid oxidation (Godwin and Prabhu 2006; Manral et al. 2008; Weber et al. 2008; Bejaoui et al., 2019). Other finding of Zhang et al (2011) showed that frying with soybean oil decreased significantly the amount of FFA and PV of fried filet.

CONCLUSION

Due to the nutritional importance of phospholipids, the present study delivers new information regarding the effects of frying on the clams' phospholipids composition. Notably, all fried flesh, especially with margarine, resulted in a significant alteration on the composition of phospholipids. These results were confirmed by a massive depletion of the nutritional quality indices and the installation of the oxidation process. Consequently, our results could be useful for providing a valuable approach for bivalves, thus ensuring that their nutritional quality is not compromised. In the future, additional investigations must be realized on the molecular characterization and identification of phospholipids overall different cooking methods and different seafood species.

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Conflict of interest

The authors declare no conflict of interest

BIBLIOGRAPHY

- Ademowo OS., Dias HKI., Milic I., Devitt A., Moran RM., Howard AN., Nolan JM., Griffiths HR., 2017. Phospholipid oxidation and carotenoid supplementation in Alzheimer's disease patients. *Free Radic Biol Med.* 108:77-85.
- Amici A., Cifuni G.F., Conto M., Esposito L., Failla S., 2015. Hunting area affects chemical and physical characteristics and fatty acid composition of wild boar (*Sus scrofa*) meat. *Rendiconti Lincei. Scienze Fisiche e Naturali.* 26: 527-534.
- Ansorena D., Guembe A., Mendizabal T., Astiasaran I., 2010. Effect of fish and oil nature on frying process and nutritional product quality. *Journal of Food Science.* 75: H62-H67
- AOCS. 1989. Official Methods and Recommended Practices of the American Oil Chemists' Society, 4th edn. Firestone, D. (Ed.). Champaign, CA: American Oil Chemists' Society. Pp. 5a-40.
- Bakar J, Zakipour RE, Cheman YB., 2008. Lipid characteristics in cooked-chill reheated fillets of Indo Pacific King Mackerel (*Scomberomorus guttatus*). *Food. Sci. Technol.* 41, 2144-2150. <https://doi.org/10.1016/j.lwt.2007.12.004>.
- Bejoui S, Rabeh I, Ghribi F, Aouini F, Chetoui I, Telahigue K, Soudani N, El Cafsi M. 2019. Change on fatty acids composition and evaluation of lipid and protein oxidation in cooked commercial clam (*Ruditapes decussatus*). *Grasas Y Aceites* 70 (4), e324. <https://doi.org/10.3989/gya.1045182>.
- Bolger Z., Brunton NP., Monahan FJ., 2017. Effect of mode of addition of flaxseed oil on the quality characteristics of chicken sausage containing vitamin E and omega 3 fatty acids at levels to support a health claim. *Food Function.* 18:33563-3575. <https://doi.org/10.1039/c7fo00929a>.
- Boselli E., Pacetti D., Lucci P., Frega NG., 2012. Characterization of Phospholipid Molecular Species in the Edible Parts of Bony Fish and Shellfish. *J. Agric. Food Chem.* 201260123234-3245
- Cui L., Decker EA., 2016. Phospholipids in foods: prooxidants or antioxidants?. *Journal of Sciences Food and Agriculture.* 15:18-31. <https://10.1002/jsfa.7320>.
- Duckett SK., Andrae JG., Owens FN., 2002. Effect of high-oil corn or added corn oil on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. *Journal of Animal Science.* 80: 3353-3360
- Echevarría N.B., Goicoechea E., Manzanos M.J., Guillén M.D., 2016. The influence of frying technique, cooking oil and fish species on the changes occurring in fish lipids and oil during shallow-frying, studied by ¹H NMR. *Food Research International.* 84: 150. DOI: [10.1016/j.foodres.2016.03.033](https://doi.org/10.1016/j.foodres.2016.03.033)
- El Reffaei WHM, Abbas NS, Atwa M, Abulhamd EM, Rasha EA., 2014. Nutritional value and fatty acid composition of household cooking on fish fatty acids profile using atherogenicity and thrombogenicity indices. *J. Food. Chem. Nut.* 02, 27-41.
- Fillion L., Henry CJ., 1998. Nutrient losses and gains during frying: a review. *Int J Food Sci Nutr.* 49:157-68.
- Foods and Wines from Spain FWS., 2017. Discover Cádiz flavors in a day. European regional development fund. <https://www.foodswinesfromspain.com/spanishfoodwine/global/food/features/feature-detail/REG2017732644.html>
- Ghribi F., Bejaoui S., Rabeh I., Aouini F., Chetoui I, ELCafsi M., 2017. Effects of culinary methods on nutritional characteristics of the edible shellfish Noah's Ark (*Arca noae* L., 1758) from Tunisian coasts. *Journal of Aquatic Food Product Technology.* <https://doi.org/10.1080/10498850.2017.1390714>
- Kinsella JE., 1990. Lipids, Membrane Receptors, and Enzymes: Effects of Dietary Fatty Acids. *Journal of Parenteral and Enteral Nutrition.* 14(55). <https://doi.org/10.1177/014860719001400511>.
- Liu Z.Y., Zhou D., Rakarlyatham K., Xie H.K., Li D.Y., Zhu B.W., Shahidi F., 2019. Impact of Frying on Changes in Clam (*Ruditapes philippinarum*) Lipids and Frying Oils: Compositional Changes and Oxidative Deterioration. *J. Am. Oil. Chem. Soc.* <https://doi.org/10.1002/aocs.12293>.
- Malavolta M., Bocci F., Boselli E., Frega NG., 2004. Normal phase liquid chromatography-electrospray ionization tandem mass spectrometry analysis of phospholipid molecular species in blood mononuclear cells: application to cystic fibrosis. *J Chromatogr B Analyt Technol Biomed Life Sci.* 25: 810(2):173-86.
- Manral, M., Pandey, M.C., Jayathilakan, K., Radhakrishna, K. & Bawa, A.S., 2008. Effect of fish (*Catla catla*) fring on the quality characteristics of sunflower oil. *Food Chemistry.* 106: 634-639.

- 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 Comp Anal* 23: 230-237. doi.org/10.1016/j.jfca.2009.10.007
- Otles G., Sengor G., 2005. Effect of various technological processes on the fatty acid composition of mussel (*Mytilus galloprovincialis*, L.). *International Journal of Food Engineering*. 1(3): 1556-3758.
- Olsen RE., Henderson RJ., 1989. The rapid analysis of neutral and polar lipids using double-development HPTLC and scanning densitometry. *J. Exp. Mar. Biol. Ecol.* 129(2): 189-197. [https://doi.org/10.1016/0022-0981\(89\)90056-7](https://doi.org/10.1016/0022-0981(89)90056-7).
- Reis A., Spickett CM., 2012. Chemistry of phospholipid oxidation. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 1818 : 2374-33287. <https://doi.org/10.1016/j.bbamem.2012.02.002>.
- Sisik SO., Akkose A., Kaban G., Kaya M., 2018. Volatile profile and fatty acid composition of kavurma (a cooked uncured meat product) produced with animal fat combinations. 1 *International Journal of Food Properties*. 21: 3364-373.
- Suja KP, Abraham TJ, Thamizh NS, Jayalekshmy A, Arumughan C., 2004. Antioxidant efficacy of sesame cake extract in vegetable oil protection. *Food Chem*. 84:393-400.
- Wang, N. ,Hatcher, D.W., Warkentin, T.D., Toews, R., 2010b. Effect of cultivar and environment on physicochemical and cooking characteristics of field pea (*Pisum sativum*). *Food. Chem.* 118(1): 109-115 <http://dx.doi.org/10.1016/j.foodchem.2009.04.082>.
- 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>.
- Wright A.C., Fan Y., Baker G.L., 2018. Nutritional value and food safety of bivalve molluscan shellfish. *Journal of Shellfish Research*. 37, 4, 695-708.
- Zhang JJ., Ji R., Hu YQ., Chen JC., Ye XQ., 2011. Effect of three cooking methods on nutrient components and antioxidant capacities of bamboo shoot (*Phyllostachys praecox* C.D. Chu et C.S. Chao). *Journal of Zhejiang University SCIENCE B*. 12: 752-759. <https://doi.org/10.1631/jzus.B1000251>.