CHANGE IN LIPIDS QUALITY AND FATTY ACIDS PROFILE OF TWO SMALL PELAGIC FISH: *SARDINELLA AURITA* AND *SARDINA PILCHARDUS* DURING CANNING PROCESS IN OLIVE OIL AND TOMATO SAUCE RESPECTIVELY

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ملخص

دراسة جودة و تركيبة الأحماض الدهنية لدى سمك السردين واللاطشة إثر عملية التحويل إلى المعلبات بزيت الزيتون و الطماطم :لدراسة تأثير عملية تحويل سمك السردين واللاطشة إلى المعلبات على الجودة الغذائية، قمنا بمتابعة موشرات جودة الدهنيات و تركيبتها من الأحماض الدهنية و أثبتت النتائج أن تغيرات كمية و جودة الدهنيات متعلقة أساسا بتركيبة سمك السردين أو اللاطشة الطازج بالدهنيات و بتركيبة المادة الحافظة (زيت الزيتون أو الطماطم). كما أثبتت التحاليل بواسطة الكروماتوغرافيا ذات الطور الغازي ثراء المعلبات بالأحماض الدهنية التالية ω9 C18 :1 و ω6 C18 :2 . وأخيرا، يمكننا التأكيد أن سمك السردين واللاطشة المعلب بزيت الزيتون و الطماطم غني بالأحماض الدهنية من عائلتي ω3 و ω6 .

الكلمات المفاتيح: تحويل، معلبات، السردين، اللاطشة، زيت الزيتون، طماطم، أحماض دهنية، أكسدة.

ABSTRACT

The effects of cooking and sterilisation steps on muscle lipid deterioration of *Sardinella aurita* and *Sardina pilchardus* canned in olive oil and tomato sauce respectively were investigated. Lipid contents of *Sardinella aurita* flesh were significantly affected ($p < 0.05$) by the canning process. However, lipid levels of *Sardina pilchardus* remained constant during processing. The peroxide value and thiobarbituric index increased slightly after the cooking step but changed significantly ($p < 0.05$) following canning. Both canned sardine and sardinella absorbed coating-oil during sterilisation inducing a higher oleic (C18:1 n-9) and linoleic (C18:2 n-6) acids content. Independently of coating-oil category, the eicosapentaenoic (C20:5 n-3) and docosahexaenoic (C22:6 n-3) acids concentrations ranged from 3.00 to 6.24% and from 5.12% to 40.26% respectively. Although, lipids of *Sardinella aurita* and *Sardina pilchardus* were slightly affected by the canning process, and they remain a good source of ω3 and ω6 fatty acids.

Keywords: Canning process; *Sardinella aurita*; *Sardina pilchardus*; olive oil; tomato sauce; polyunsaturated fatty acids; lipids; oxidation.

RÉSUMÉ

Variation de la qualité et de la composition en acides gras des lipides de deux petits poissons pélagiques : *Sardinella aurita* et *Sardina pilchardus* au cours de la mise en boite dans l'huile d'olive et la sauce de tomate : Dans le présent travail, les effets de la cuisson et de la stérilisation sur la qualité et la composition en acide gras des lipides de la sardinelle *Sardinella aurita* et la sardine *Sardina pilchardus* au cours de la mise en boite dans l'huile d'olive et la sauce de tomate ont été étudiés. La teneur en lipides dans la chair de la sardinelle *Sardinella aurita* a augmenté significativement ($p <0.05$) après la mise en boite. Cependant, celle de la sardine *Sardina pilchardus* a demeuré stable dans les mêmes conditions. D'autre part, l'étude des indicateurs d'altération des lipides nous a montré que la qualité des lipides de la sardine est affectée par la stérilisation mais pas par la cuisson.

La composition en acides gras de la sardine et la sardinelle en boite de conserve change significativement après stérilisation. En effet, indépendamment de la nature d'huile conservatrice, les lipides de la matière première s'enrichissent en acide oléique (C18:1 n-9) et linoléique (C18:2 n-6). Les lipides de la sardine et la sardinelle en boite représentent une source en acides gras poly insaturés de la familles des ω3 et ω6.

Mots clés: Mise en boite; *Sardinella aurita*; *Sardina pilchardus*; huile d'olive; Sauce tomate; Acide gras polyinsaturés; Lipides ; Oxydation.

INTRODUCTION

Lipids from marine source are considered to have several health enhancing properties, largely attributed to their vitamin and n-3 fatty acid contents (Aidos *et al*. 2002). Research studies revealed that the consumption of fish increases the levels of ecosapentaenoic acid EPA and docosahexaenoic acid DHA in blood which reduce the rate of coronary heart diseases via different actions (Sidhu, 2003). The antiathrogenic and antithrombotic effects of omega-3 fatty acids demote the growth of lipid rich atherosclerotic plaques and decrease the risk of thrombosis inhibing the formation of thrombus (Schacky, 2000 ; Schmidt *et al*. 2005). It has been found that fish oil also exert their protective effect against, lowering blood pressure, and decreasing inflammation (Kris-Etherton *et al.* 2003). Christensen *et al*. (1997) found that dietary n-3 fatty acids act to prevent arrhythmias that can lead to sudden cardiac death.

Several studies have reported changes in lipid during thermal treatment of fish such as cooking (Aubourg *et al*. 1997), smoking (Stołyhwo *et al*. 2006) and frying (Sebedio *et al.* 1993). In the case of the canning process, the fatty acid and lipid class compositions have been studied (Tarley *et al*. 2004) as well as the quality of the final product as a function of the packaging method and the storage temperature of the raw material. It is well established that during high temperature process, damage to polyunsaturated fatty acids can lead to primary and secondary lipids oxidation products (Maruf *et al*. 1990 ; Boran *et al*. 2006). Lipid oxidation compounds appear during the cooling step of cooked fish, which may influence the quality of canned sardine. Thus, the effect of canning on chemical composition in marine products has been extensively investigated. Nevertheless, information about lipids changes produced from the earlier steps of processing are rather scarce. The aim of this study was to determine the effect of canning process and coating-oil nature on lipids sardine flesh by fatty acids profiles and lipids quality indicators.

MATERIALS AND METHODS

1- Fish sample and processing

Samples of fresh, cooked and canned small pelagic fish *(Sardina pilchardus* and *Sardinella aurita)* were obtained from the industrial tuna and sardine processing Unit (ABCO) in Tunisia (*S. pilchardus* in February 2005 and *S. aurita* in April 2007). During processing, samples were gutted, headed then subjected to the different stage of canning process described in figure 1.

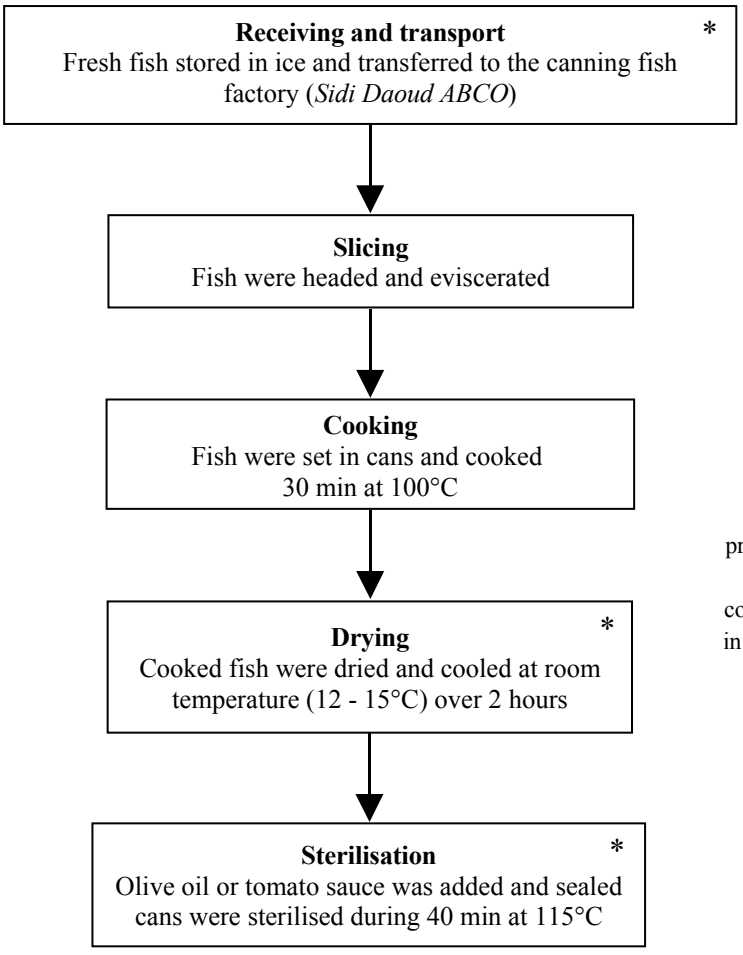

Figure 1 : Flow diagram of the production of *Sardinella aurita* and *Sardina pilchardus* cans. * correspond to tissue sampling (n= 9 in each time) at different processing

Samples were canned either with a covering olive oil and tomato sauce, and the sampling (n = 9 in each step) was performed on fresh fish, following the cooking and sterilisation steps. Cans (120 g) from each species belonging to the same lot of processed fish, were opened and the liquid was drained off carefully, the muscle part was pooled, minced and enclosed in filter paper to absorb free liquid before analysis.

2- Lipids extraction

For the total lipid extraction, 3g of tissue (n = 6 for each species and in each sampling step) were extracted according to the method of Bligh and Dyer, (1959) by chloroform/methanol (2/1), the lipid fraction was determinate gravimetrically.

3- Fatty acids analysis

Fatty Acids Methyl Esters (FAMEs) were obtained by the method described by Metcalfe *et al*. (1966). A fraction of lipids extract was saponified with 0.5N NaOH in methanol followed by methylation in 14% Boron trifluoride in methanol (BF_3/MeOH). The methylated sample was then extracted with n-hexane. All of these reactions were performed in quadruplet for each sample.

The resulting methyl esters were analysed by gas chromatography (GC) using an Agilent technologies chromatograph 6890N equipped with a flame ionization detector (FID), a splitless injector and a polar INNOWAX 30 M silica capillary column (0.25 mm i.d * 30m length * 0.25 µm film thickness). The temperature of the injector and detector were 220 and 275°C respectively. Helium was used as a carrier gas with a flow rate of 1.5 ml/mn. Peaks were identified by comparison of their retention times with FAMEs standards (SUPELCO PUFA-3). The sequences of fatty acids ranged according to their chromatographic retention times and the values are given as percentages of the total fatty acids methyl esters.

4- Peroxide value (PV)

The PV was determined by the measurement of iron oxidation according to Shantha & Decker method (1994). The content was expressed in terms of meq of active O_2 per kg of lipid.

5- Thiobarbituric acid index (TBArs)

The TBArs was determined according to the AOCS method (1998). This procedure permits the direct determination of TBArs in oils and fats without preliminary isolation of secondary oxidation products; it is applicable to animal and vegetable fats and oils. The results were expressed as mg malonaldehyde/kg of oil.

6- Statistical Analysis.

Statistical analysis was performed using SPSS software version 10.0.5. The comparison of lipid content, fatty acid compositions, PV and TBArs between processing stages were tested using Duncan's test (95% confidence interval) with one-way ANOVA. Data are expressed as mean ± standard error; n values are given for each table and figure.

RESULTS AND DISCUSSION

1- Fat analysis

The initial lipid contents found in this study in flesh samples *Sardinella aurita* and *sardina pilchardus* were 6.28 and 1.16 g/100g respectively. The low lipid levels found for both sardine species were related to season. Thus, it is well known that small pelagic fish like *Sardina pilchardus* are fatty species with levels reaching 18.2% (Bandarra *et al*. 1997), such levels are however affected by the catching season and the fishing area (Aidos *et al*. 2002 ; Njinkoue *et al*. 2002). The cooking process has no significant effect ($p > 0.05$) on the lipids content of both sardine species. Subsequently after olive oil addition and sterilisation, total fat increased significantly in the *Sardinella aurita* flesh to reach 8.62g/100g; this increase due to the incorporation of olive oil into the muscle.

Contrasting with the results found for canned *S. aurita* in olive oil, lipid content from *Sardina pilchardus* in tomato sauce cans showed a non-significant variation after the sterilisation process. Such result showed that although *S. pilchardus* was coated in tomato sauce, which is rich in rape oil, lipid content was not affect.

2- Fatty acids composition

The fatty acids compositions of both sardine species (*Sardinelle aurita* & *Sardina pilchardus*) flesh sampled at each stage of the canning process and coated in olive oil or tomato sauce samples are presented in Tables (I and II). The sequences of fatty acids were ordered according to their chromatographic retention times and their concentrations were given as percentages of the total fatty acids methyl esters.

In fresh *Sardina pilchardus*, polyunsaturated fatty acids (PUFAs) constitute the majority of the fatty acids pool followed by saturated fatty acids (SFAs), and monounsaturated fatty acids (MUFAs). Within these groups, the major fatty acids were palmitic acid (C16:0), oleic acid (C18:1 n-9 & n-7), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

In the case of fresh *Sardinella aurita*, fatty acids profile show a different composition (Table 2) with significantly higher SFAs content and a lower PUFAs fraction then that of *sardina pilchardus*.

Cooking process had a significant effect ($p < 0.05$) on saturated and monounsaturated fatty acids composition in *Sardina pilchardus* and *Sardinella aurita*. The content of C16:0 and C18:1 n-9, was significantly changed; however, polyunsaturated fatty acids in *Sardina pilchardus* were not influenced by such processing. This result was in agreement with

Table I : Changes in fatty acids profile of *Sardinella aurita* flesh during cooking and canning process in olive oil. Values are proportions (%) of total fatty acids. Means (n = 4) with the same letter within rows are not significantly different (p > 0.05). *, **, and *** represent significance at 0.05, 0.01, and 0.001 respectively. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Fatty acids	Fresh sample	Cooked sample	Canned sample	Olive oil	AV
C14:0	11,71 ±0,84 a	13,00 ±0,16 a	7,34 ±0,13 b	0,05 ± 0,01 c	***
C14:1	0,56 ±0,09 a	0,82 ±0,05 a	0,59 ±0,20 a	-	
C15:0	2,04 ±0,10 a	2,16 ±0,18 a	1,28 ±0,02 b	0,01 ± 0,01 c	***
C15:1	0,27 ±0,04 a	0,29 ±0,01 a	0,11 ±0,01 b	-	**
C16:0	36,27 ±0,36 a	38,70 ±0,27 b	33,96 ±0,54 c	15,28 ± 0,00 d	***
C16:1 ω7	7,03 ±0,33 a	7,38 ±0,08 a	5,42 ±0,19 b	2,05 ± 0,00 c	***
C16:2 ω4	0,43 ±0,04 a	0,49 ±0,03 a	0,16 ±0,01 b	-	**
C17:0	0,45 ±0,04 a	0,56 ±0,01 a	0,46 ±0,04 a	0,06 ± 0,00 b	*
C17:1	0,22 ±0,04 a	0,19 ±0,00 a	0,17 ±0,02 a	-	
C18:0	2,97 ±0,07 a	3,32 ±0,02 a	3,24 ±0,30 a	2,37 ± 0,03 b	*
C18:1 ω9	14,62 ±1,75 a	10,81 ±0,09 b	24,30 ±0,38 c	58,72 ± 0,11 d	***
C18:1 ω7	1,58 ±0,11 a	1,67 ±0,07 a	0,09 ±0,05 b	3,25 ± 0,03 c	***
C18:2 ω6	4,16 ±0,69 a	2,45 ±0,06 b	7,94 ±0,06 c	16,87 ± 0,05 d	***
C18:3 ω3	1,48 ±0,06 a	1,61 ±0,01 a	1,23 ±0,07 b	-	**
C20:0	0,13 ±0,01 a	0,06 ±0,03 a	0,13 ±0,00 a	0,42 ± 0,00 b	*
C20:2	0,12 ±0,04 a	0,04 ±0,02 b	0,03 ±0,01 b	-	*
C20:3	0,37 ±0,02 a	0,06 ±0,03 b	0,04 ±0,02 b	-	*
C20:4 ω3	0,49 ±0,03 a	0,59 ±0,00 b	0,41 ±0,01 c	-	**
EPA C20:5 ω3	3,92 ±0,25 a	4,36 ±0,06 a	3,00 ±0,15 b	0,06 ± 0,01 c	***
C22:5 ω3	0,49 ±0,05 a	0,45 ±0,07 a	1,17 ±0,22 b	-	***
DHA C22:6 ω3	6,09 ±0,18 a	6,80 ±0,08 b	5,12 ±0,04 c	0,33 ± 0,03 d	***
SFA	53,57 ±1,24 a	57,81 ±0,55 b	46,40 ±0,43 c	18,30 ± 0,07 d	***
MUFA	24,27 ±1,31 a	21,18 ±0,08 b	30,67 ±0,51 c	64,02 ± 0,14 d	***
PUFA	17,54 ±0,33 ab	16,85 ±0,05 a	19,09 ±0,35 b	17,26 ± 0,09 ab	**
EPA/DHA	0,64 ±0,02 a	0,64 ±0,00 a	0,59 ±0,03 a	0,18 ± 0,33 b	**
PUFA/SFA	0,33 ±0,01 a	0,29 ±0,01 b	0,41 ±0,00 c	0,94 ± 1,29 d	***
Lipids (%)	6,28 ±0,57 a	6,77 ±0,62 a	8,62 ±0,40 b	-	***

those of Aubourg *et al*. (1997) who reported that PUFAs in tuna lipid were not influenced by the cooking process.

SFAs, MUFAs and PUFAs profiles in the lipids flesh of both sardines species changed significantly ($p < 0.05$) after the canning process in olive oil and tomato sauce to attain 46.40; 30.67; 19.09% in *S. aurita* and 27.58; 23.05; 41.92% in *S. pilchardus*. Within the PUFAs group, the content of EPA and DHA decreased to reach 3.00; 5.12% and 4.15; 25.79% in *Sardina pilchardus* and *Sardinella aurita* respectively. A higher content of the essential C18:1 n-9 and C18:2 n-6 fatty acids were found in the canned samples than in fresh and cooked samples. Canned sardines were characterised by their richness in oleic (21.52-24.39%) and linoleic (10.54-7.94 %) acids after the sterilisation process. The high concentration of C18:1

Table II : Changes in fatty acids composition of *Sardina pilchardus* flesh during cooking and canning process in tomato sauce. Values are proportions (%) of total fatty acids. Means (n = 4) with the same letter within rows are not significantly different (p > 0.05). *, **, and *** represent significance at 0.05, 0.01, and 0.001 respectively. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Fatty acids	Fresh sample	Cooked sample	Canned sample	Tomato sauce	AV
C14:0	4,50 ± 0,19 a	1,66 ± 0,12 b	1,46 ± 0,11 b	2,53 ± 0,09 c	***
C14:1	0,20 ± 0,01 ab	0,31 ± 0,17 b	0,06 ± 0,01 a	0,13 ± 0,00 ab	NS
C15:0	1,05 ± 0,05 a	0,49 ± 0,21 b	0,47 ± 0,03 b	0,51 ± 0,01 b	**
C15:1	0,34 ± 0,03 a	0,35 ± 0,16 a	0,10 ± 0,01 b	0,07 ± 0,00 b	*
C16:0	25,51 ± 0,94 a	26,29 ± 0,94 a	20,34 ± 0,09 b	14,39 ± 0,31 c	***
C16:1 ω7	2,00 ± 0,06 a	0,86 ± 0,16 b	1,37 ± 0,03 c	1,81 ± 0,04 d	***
C16:2 ω4	0,23 ± 0,02 a	0,14 ± 0,01 b	0,10 ± 0,00 c	0,12 ± 0,00 bc	***
C17:0	0,58 ± 0,07 a	1,12 ± 0,10 b	0,72 ± 0,03 a	0,65 ± 0,01 a	NS
C18:0	5,01 ± 0,16 a	4,98 ± 0,14 a	4,22 ± 0,05 b	3,38 ± 0,04 c	***
C18:1 ω9	6,96 ± 0,22 a	6,15 ± 0,16 b	19,57 ± 0,07 c	14,13 ± 0,18 d	***
C18:1 ω7	1,85 ± 0,06 a	1,64 ± 0,06 a	1,95 ± 0,00 a	1,45 ± 0,02 a	NS
C18:2 ω6	1,46 ± 0,05 a	1,30 ± 0,06 a	10,54 ± 0,03 b	23,62 ± 0,34 c	***
C18:3 ω3	1,09 ± 0,04 ab	0,70 ± 0,03 b	1,47 ± 0,01 a	3,77 ± 0,05 c	***
C20:0	0,37 ± 0,04 a	0,32 ± 0,03 a	0,36 ± 0,03 a	0,25 ± 0,01 a	NS
C20:4 ω3	1,39 ± 0,02 a	1,05 ± 0,11 b	0,89 ± 0,00 c	0,20 ± 0,01 d	***
EPA C20:5 ω3	6,24 ± 0,19 a	4,93 ± 0,14 b	4,15 ± 0,08 c	3,46 ± 0,01 d	***
DHA C22:6 ω3	33,61 ± 1,01 a	40,26 ± 0,48 b	24,79 ± 1,79 c	7,25 ± 0,05 d	***
SFA	37,00 ± 0,97 a	34,48 ± 0,95 b	27,58 ± 0,07 c	21,54 ± 0,37 d	***
MUFA	11,34 ± 0,27 a	9,26 ± 0,07 b	23,05 ± 0,11 c	17,49 ± 0,19 d	***
PUFA	44,03 ± 0,92 a	48,46 ± 0,59 b	41,92 ± 1,48 a	40,21 ± 1,81 a	**
EPA/DHA	0,19 ± 0,00 a	0,12 ± 0,00 a	0,17 ± 0,01 a	0,41 ± 0,06 b	***
PUFA/SFA	1,19 ± 0,01 a	1,40 ± 0,05 b	1,52 ± 0,05 b	1,79 ± 0,10 c	***
Lipids (%)	1,16 ±0,16 a	1,24 ±0,18 a	1,28 ±0,15 a	-	***

and C18:2 fatty acids and the lower EPA and DHA values, when compared to fresh samples, may be explained by the incorporation of the coating media into the flesh. Thus, the analysis of the coating media (olive oil and tomato sauce) revealed high levels of C18:1 n-9 (61.97%) and C18:2 n-6 (23.62%) fatty acids respectively. Similar results were reported in other studies (Aubourg *et al*. 1996 ; Badolato *et al*. 2003). It is suggested that such enrichment enhance the nutritional oil quality of the sardine flesh *Sardinella brasiliensis* (Tarley *et al*. 2004).

3- Peroxide value (PV) and thiobarbituric acid (TBArs)

Fats rich in polyunsaturated fatty acids, are very sensitive to oxidation. TBArs and PV measurements were performed during the cooking and sterilisation process (Table III).

The hydroperoxide values of cooked and canned *Sardinella aurita* (12.78 - 14.27 meq active O_2/kg oil respectively) were higher than that of fresh samples (10.230 meq active O_2/kg oil). Consequently, the increase of TBArs after the cooking process may be explained by the action of aqueous radicals that are abundant in the flesh fish rich in water. However, the

Table III : Changes in peroxide value (PV) and thiobarbituric acid index (TBArs) *Sardinella aurita* and *Sardina pilchardus* lipids flesh during the cooking and canning process. PV and TBArs are expressed in terms of meq of active O_2 per kg of oil and

mg malonaldehyde/kg of oil respectively. Means (n = 4) with the same letter within rows are not significantly different ($p < 0.05$). *, **, and *** represent significance at 0.05, 0.01, and 0.001, respectively. 3M: three months.

		Fresh sample	Cooked sample	Canned sample 3M	AV
Sardinella aurita	PV	10,23 ± 0,55 a	12,78 ± 0,78 b	14,27 ± 0,43 c	***
	TBArs	1,49 ± 0,18 a	3,17 ± 0,53 b	1,15 ± 0,21 a	***
Sardina pilchardus	PV	8,14 ± 0,49 ab	10,18 ± 1,14 b	8,19 ± 0,34 a	*
	TBArs	0,87 ± 0,21 a	1,13 ± 0.15 a	1,94 ± 0.21 b	**

Sardina pilchardus PV levels changed but not significantly during the cooking step. Our results may be explained by the lower lipids content in fresh *Sardina pilchardus* (1.16%).

PV of all examined samples did not exceed 20 meq O_2/kg oil with a measured maximum value of 14.27 meq O_2/kg oil in canned *Sardinella aurita*. In the first stage of canning process, TBArs values of both sardines samples showed the same tendency as those of PV, then declined to reach 1.15 and 1.94 mg mal/kg oil after the canning process of *Sardinella aurita* and *Sardina pilchardus* respectively. The decrease TBArs levels, may be explained by the diluting effect of the coating oil on secondary oxidation products and/or by the thermal treatment effect during the cooking process. It was stated that during cooking, TBArs can react with other biological compound generating substances with fluorescent properties (Maruf *et al*. 1990).

CONCLUSION

The difference between fresh, cooked and canned both sardine species *(Sardinella aurita* and *Sardina pilchardus)* was related to the initial fatty acids composition, the heating process and the coating-oil medium (olive oil or tomato sauce). Lipid flesh were slightly affected by the canning process, therefore, we can assume that canned small pelagic fish in olive oil and tomato sauce are a good source of ω3 and ω6 fatty acids (oleic and linoleic acids, EPA and DHA).

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