

Effects of Drying Methods on the Chemical Composition of the Sea Cucumber *Holothuria forskali*

Khaoula Telahigue^{1,*}, Tarek Hajji^{1,2}, Rabeh Imen¹, Ouertani Sahbi³ and M'hamed El Cafsi¹

¹University of Tunis El Manar, Faculty of Sciences of Tunis, Research Unit of Physiology and Aquatic Environment, 2092 Tunis, Tunisia

²University of Manouba, High Institute of Biotechnology of Sidi Thabet, 2020 Ariana, Tunisia

³University of Tunis El Manar, Faculty of Sciences of Tunis, Laboratory for Energy and Heat and Mass Transfer, 2092 Tunis, Tunisia

Abstract: The effects of artisanal and controlled oven drying methods on the biochemical composition of the body wall of the sea cucumber, *Holothuria forskali* were investigated. Five combinations of temperature (°C) and relative humidity (%) levels were tested (50°C, 20%, 60°C, 25%; 60°C, 15%, 60°C, 20% and 70°C, 20%). The results demonstrate that the treatment (60°C and 20%) yields an improvement of the nutritional quality of the initial product. Improvements were particularly noted in terms of the amount of total proteins and the polyunsaturated fatty acids level in the dry body wall of *H. forskali*.

Keywords: Chemical composition; fatty acid; *Holothuria forskali*; oven drying; solar drying; Tunisia.

1. INTRODUCTION

The sea cucumbers belong to the phylum of Echinodermata. They are commonly known as trepang, bêche-de-mer, or gamat. The sea cucumbers have high nutritional values with a high quality protein and low fat levels [1, 2]. They are also traditional tonic and widely consumed in East Asian countries [3].

From medicinal perspective, some sea cucumbers species are used in traditional medicine in Asia and in the Middle East to treat wounds, eczema, arthritis and hypertension [4]. Several bioactive compounds of trepan have unique biological and pharmacological activities that were reported in numerous studies. These compounds exhibit antibacterial [5], antifungal [6, 7] and antioxidant [8] properties. Furthermore, previous studies revealed that some of sea cucumber species contain potential anti-tumor and immunostimulatory agents [9-11].

Since sea cucumbers can autolyze after they are taken out of sea water, they are mostly processed into dried, boiled or salted products [12, 13].

Traditional techniques, which involve salting, repeated boiling and exposure to solar radiation, are long processes that lead to the loss of many active ingredients [3]. In order to yield better quality of dried sea cucumbers, new drying technologies are used. Hot air drying is the simplest method,

but it leads to significant changes in quality of the products [13-15]. In this respect, the most determinant drying factors to avoid the deterioration of the final product are temperature, humidity and air velocity. Therefore, these three parameters must be optimized for a better quality of the dried product.

Studies relating to the effects of cooking types on fatty acid composition of sea food have been mainly focusing on fish frying and boiling [16-20]. Little information exists regarding the effect of drying on the fatty acid profile of seafood, including sea cucumbers.

The objective of this study is to examine the effects of artisanal and controlled drying methods on the biochemical composition and fatty acids profile of the body wall of *Holothuria forskali* that are collected in Tunisian coasts.

2. MATERIAL AND METHODOLOGY

2.1. Materials

Two hundred and ten (210) live specimens of sea cucumbers *Holothuria forskali* (average body weight 100g) were hand-picked by scuba diving (Fig. 1 below shows the harvesting sites of sea cucumbers). They were collected from the Northern East coast of Tunisia in the locality of Bizerte.

2.2. Pretreatment

The sea cucumbers were transported to laboratory in seawater. The body of each specimen was cut from the anus nearly to the oral organ, and then their viscera were

*Address correspondence to this author at the University of Tunis El Manar, Faculty of Sciences of Tunis, Research Unit of Physiology and Aquatic Environment, 2092 Tunis, Tunisia; Tel: +21623312023; Fax: +21671537044; E-mail: k_telahigue@yahoo.fr

removed. The body walls were washed carefully with distilled water.

2.3. Drying Trials

2.3.1. Solar Drying

After a 45 minutes boiling time in sea water , the body walls of eviscerated sea cucumbers were drained, salted and sun dried for few days until “stone dried” (four to five days).

2.3.2. Oven Drying

Controlled drying was undertaken through a convective hot air drying processing system (Fig. 2) [21]. The unit was composed of a centrifugal blower to aspire the ambient air,

an electrical resistance for air heating, a heating control unit, a steam air moistening section, the sensors and a drying chamber. A high precision balance was installed at the lower part of the drier. A controlled automated system was used to adjust temperature, air velocity and relative humidity.

The measurement sensors and the data recording and controlling systems are connected to a computer. Hot air was vertically orientated on the samples to ensure optimum conditions for air-product contact.

To assess optimal environment for the sea cucumber drying, five conditions were evaluated. They are detailed in the Table 1.

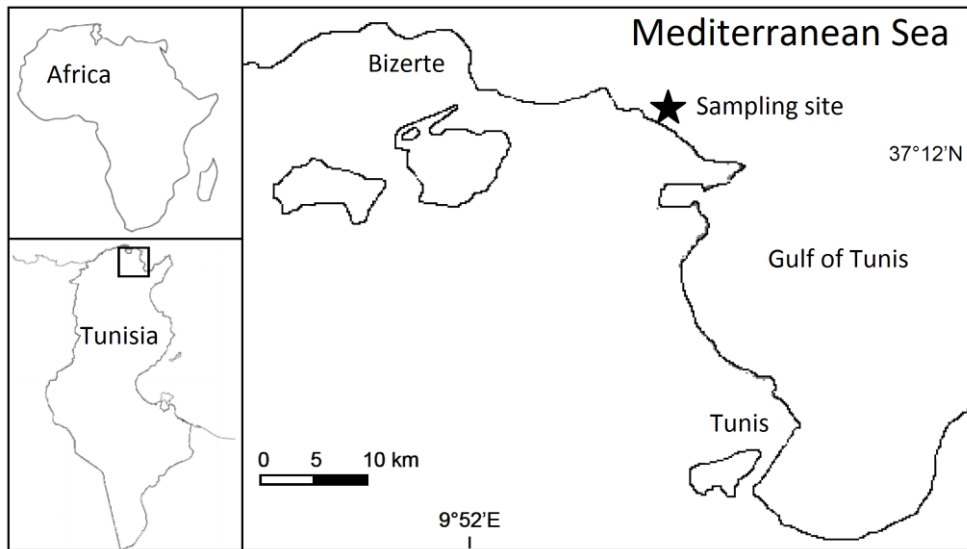


Fig. (1). Sampling site located in the Northern East cost of Tunisia.

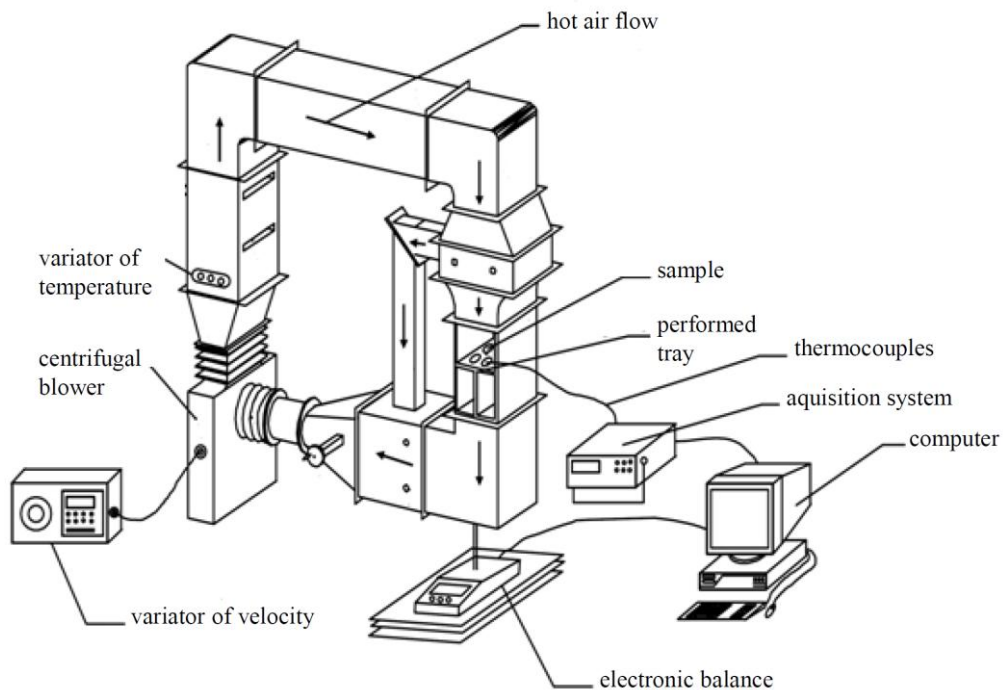


Fig. (2). Dryer system (Hassini et al. [21]).

Table 1. Experimental Conditions of Drying Processes. Treat: Treatment

Treatment	Temperature (°C)	Relative Humidity (%)	Air Velocity (m/s)
Treat.1	50	20	2
Treat.2	60	25	2
Treat.3	60	15	2
Treat.4	60	20	2
Treat.5	70	20	2

2.4. Analysis of Sample

2.4.1. Moisture Content

Moisture content wet basis [M.C. (w.b.)] was determined by oven drying at 105°C for 24h using the following formula according to Ranganna [22]:

$$MC(w.b.) = \frac{w_1 - w_2}{w_1} * 100$$

Where,

w_1 : weight of sample before drying and w_2 : weight of dried sample.

2.4.2. Protein Estimation

Protein concentration was determined as described by Lowry *et al.* [23] with bovine serum albumin (BSA) as standard.

2.4.3. Total lipid Extraction

Lipids were extracted according to the Folch method [24] with the chloroform-methanol solvent mixture (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as the antioxidant.

2.4.4. Fatty acid Analysis

After evaporation to dryness, lipid extracts were transesterified according to the method of Cecchi [25]. Separation of FAMES was carried out on a HP 6890 gas chromatograph with a split/splitless injector equipped with a flame

ionization detector at 275°C, and a 30 m HP Innowax capillary column with an internal diameter of 250 μ m and a 0.25 μ m film thickness. Injector temperature was held at 250°C. The oven was programmed to rise from 50 to 180°C at a rate of 4 C/min, from 180 to 220°C at 1.33 C/min and to stabilize at 220°C for 7 minutes. The carrier gas was nitrogen.

Identification of FAMES was based on the comparison of their retention times with those of a mixture of methyl esters (SUPELCO PUFA-3). Fatty acid peaks were integrated and analyzed using the HP chemstation software.

All chemical analyses were performed in triplicate. The results were expressed in dry terms (g/100g dry weight) for protein and fat. Fatty acid contents were expressed as percentages of total fatty acids. Data were presented by calculating the mean value \pm standard deviation.

2.5. Statistical Analysis

Data was analyzed using the software "R". Values are presented as mean \pm SD. To assess significant differences between means, the one way Analysis of Variance (ANOVA) followed by the Duncan test was applied. Differences were considered significant when $P < 0.05$.

3. RESULTS

Fig. (3) relates the moisture content of sea cucumber body wall to drying time for different oven treatments. The curve of best fit for the drying process was recorded for the

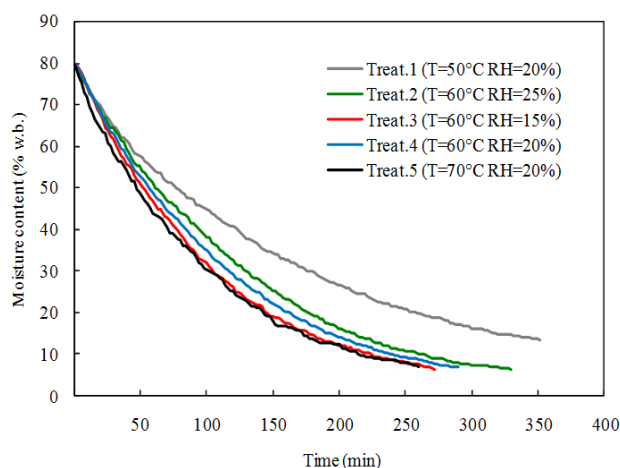


Fig. (3). Variations of the moisture content of the sea cucumber body wall vs. drying time obtained at different drying treatments.

body wall that was exposed to a 70°C temperature and a 20 % relative humidity (Treat.5). This condition allows to gain 12 minutes of drying time in comparison with Treat.3, 30 minutes with the Treat.4, 70 and 100 minutes with the Treat.2 and Treat.1, respectively.

The effect of different drying treatments on protein content in the body wall of *H. forskali* is given in the Fig. (4). Compared to the raw samples, results show that the oven treatments and sun-drying significantly increase protein content ($p < 0.05$). The highest protein content was reported in the sea cucumber processed with the Treat.5 that reached 52.4% of the dry weight.

As reported in Fig. (5), the raw body wall of *H. forskali* appeared to be relatively low in fat (0.40%). This value was insignificantly decreased ($p > 0.05$) by the sun-drying (0.34%). However, we noted that different oven treatments induced a significant raise of fat content ($p < 0.05$). The highest increases (0.66%, 0.67 % and 0.63%) are respectively recorded for three conditions under a relative

humidity of 20% coupled with a 50°C, 60°C and 70°C temperature levels.

The fatty acids compositions of the raw and dried body wall of *H. forskali* are given in Table 2. Fatty acids present in the raw body wall at 5% or more of the total fatty acids were: C14:0, C16:0, C18:0, C18:1n-7, C18:1n-9, C22:1 and C16:3.

Different drying treatments (sun and oven) induced a significant increase ($p < 0.05$) of the sum of saturated fatty acids (Σ SFA). We noted that the fifth oven drying treatment (Treat.5: 70°C, 20%) exhibits the highest value (76.9%). These results were the consequence of the strong increase of the C16:0 and the C18:0 that were recorded at different drying processes. The sum of monounsaturated fatty acids (Σ MUFA), shows a decline ($p < 0.05$) in the solar-dried and the oven dried body walls.

The sum of polyunsaturated fatty acids (Σ PUFA) shows different trends along the drying processes. Dried samples

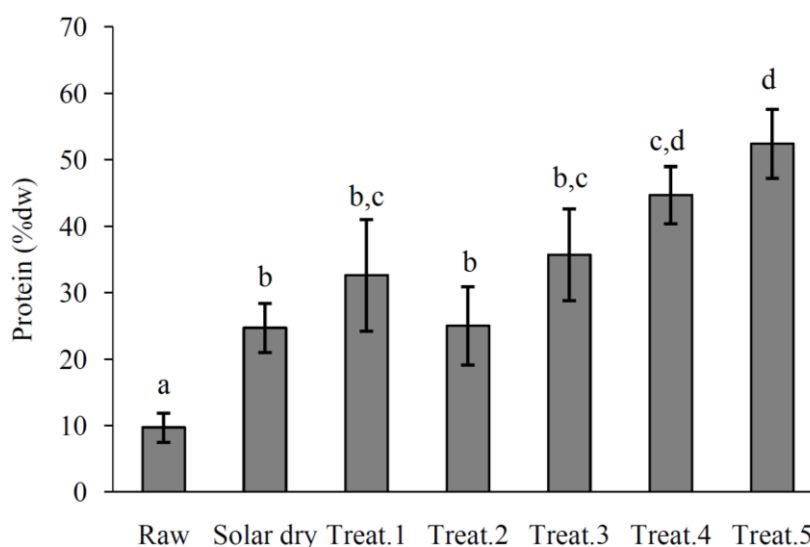


Fig. (4). Total protein content (g/100g) in the raw and dried body wall of the sea cucumber *Holothuria forskali*. Different letters indicate significantly different values (Duncan's $p < 0.05$) between treatments.

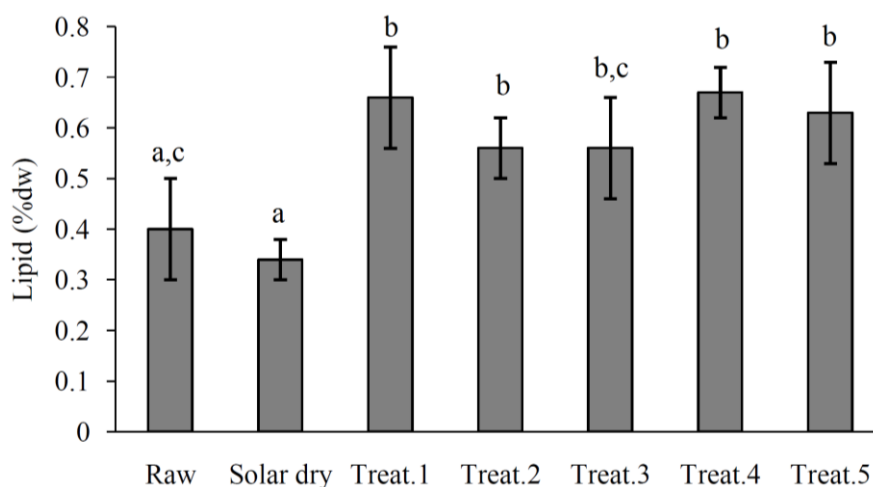


Fig. (5). Total lipid content (g/100g) in the raw and the dried body wall of the sea cucumber *Holothuria forskali*. Different letters indicate significantly different values (Duncan's $p < 0.05$) between treatments.

with Treat.1 and Treat.5 were significantly ($p<0.05$) lower in total polyunsaturated fatty acids (16.1% and 17.3%, respectively) than the raw sample (23.7%). However, the Σ PUFA was not significantly affected ($p>0.05$) by the sun drying (19.5%) and by the oven drying for Treat.3 (27.6%), Treat.2 (21.3%) and Treat.4 (23.7%).

Among the PUFAs group, results show that the n-3 fatty acid content (as % of total fatty acids) in raw bêche-de-mer was 4.1%. Different drying methods led to significant increase in total n-3PUFA ($p<0.05$). The highest value (13.8%) was recorded for Treat.2. This treatment shows also the highest docosahexaenoic acid (C22:6n-3, DHA) and

eicosapentaenoic acid (C20:5n-3, EPA) contents (2.3% and 6.2%, respectively) when compared with the other samples.

Concerning the (n-6) PUFAs, results show that the dihomo-gamma-linolenic acid (C20:3n-6) was the major component of this series in different analyzed samples except for those dried with Treat.3. These revealed an interesting elevation ($p<0.05$) of the arachidonic acid (ARA, C20:4n-6) which reached 4.8%.

When calculating the ratio of omega-3 to omega-6 fatty acids, we noted that different drying methods led to a significant increase of this ratio. Values range from 0.6 in the raw body wall to 3.0 and 5.8 for Treat.2 and Treat.5.

Table 2. Fatty Acid Composition of Total Lipids Extracted from the Body Wall of the sea Cucumber *Holothuria forskali* (n=6)

Fatty acid	Raw Mean±SD	Solar Dry Mean±SD	Oven Dry				
			Treat.1 Mean±SD	Treat.2 Mean±SD	Treat.3 Mean±SD	Treat.4 Mean±SD	Treat.5 Mean±SD
C14:0	15.8±3.8	4.2±0.8	5.2±1.6	4.5±0.6	4.3±0.7	4.8±1.3	3.6±0.9
C16:0	13.8±3.6	28.7±7.4	31.2±3.8	30.0±1.4	28.7±5.6	29.6±3.6	31.3±4.2
C18:0	13.2±3.0	35.6±1.8	34.1±2.8	22.2±1.3	30.3±6.3	33.1±2.5	42.0±0.9
Σ SFA	42.9±2.9	68.5±9.5	70.5±6.1	56.7±1.7	63.3±4.6	67.5±5.1	76.9±1.7
C16:1n-7	2.5±0.6	2.7±0.6	3.0±0.4	5.3±0.9	1.5±0.4	1.2±0.4	1.4±0.7
C18:1n-7	13.2±2.8	1.1±0.7	1.9±0.9	8.7±2.4	1.2±0.1	1.4±0.3	0.5±0.1
C18:1n-9	5.6±1.2	2.2±1.1	2.3±0.3	3.2±0.4	2.3±0.7	1.7±0.1	0.8±0.2
C20:1	2.5±0.5	1.1±0.4	1.4±0.6	0.5±0.1	1.0±0.3	0.9±0.1	0.7±0.3
C22:1	9.7±3.2	4.8±1.8	4.9±0.9	4.4±1.7	3.1±1.1	3.6±0.9	2.5±0.9
Σ MUFA	33.4±1.6	12.0±1.6	13.4±0.6	22.1±3.6	9.1±0.6	8.8±2.7	5.8±0.9
C16:2	4.4±1.4	1.3±0.3	0.3±0.0	0.3±0.1	1.6±0.3	0.6±0.1	0.2±0.0
C16:3	8.3±0.7	1.6±0.2	1.8±0.7	1.3±0.5	2.1±0.4	1.7±0.7	0.2±0.1
C16:4	0.4±0.1	1.7±0.9	1.4±0.4	1.4±0.9	2.2±0.7	1.9±0.5	1.4±0.3
C18:3n-3	1.8±0.4	1.4±0.4	2.3±1.1	5.4±1.4	7.8±2.4	9.9±1.1	10.6±1.2
C20:5n-3	1.4±0.4	5.7±0.9	4.8±1.3	6.2±0.6	1.8±0.9	1.7±0.3	1.9±0.6
C22:6n-3	0.9±0.1	1.8±0.2	1.4±0.3	2.3±0.9	2.1±0.3	1.6±0.1	0.7±0.1
Σ (n-3)	4.1±0.3	8.8±0.7	8.4±0.9	13.8±0.8	11.7±2.0	13.2±2.9	13.2±3.4
C18:2n-6	0.4±0.0	1.0±0.3	1.2±0.5	1.1±0.8	1.1±0.2	0.8±0.2	0.2±0.0
C20:2n-6	1.9±0.4	1.5±0.9	0.7±0.1	1.1±0.2	1.1±0.0	1.0±0.1	0.6±0.2
C20:3n-6	4.1±1.2	3.2±0.5	2.3±0.3	2.2±0.2	3.1±0.6	2.4±0.8	1.2±0.4
C20:4n-6	0.2±0.0	0.3±0.1	-	0.2±0.0	4.8±0.3	0.9±0.1	0.3±0.0
Σ (n-6)	6.5±0.8	6.1±0.3	4.2±1.2	4.6±0.9	10.1±2.8	5.1±1.6	2.3±0.4
Σ PUFA	23.7±8.6	19.5±3.7	16.1±1.9	21.3±2.9	27.6±1.2	23.7±3.5	17.3±2.6
n-3/n-6	0.6±0.0	1.4±0.1	2.0±0.6	3.0±1.1	1.2±0.4	2.6±0.6	5.8±0.8

Data are expressed as mean percentages of total fatty acids. SAFA: sum of saturated fatty acids, MUFA: sum of monounsaturated fatty acids, PUFA: sum of polyunsaturated fatty acids, Treat: treatment.

4. DISCUSSION

Biochemical analysis of the raw and the dried body wall of *H. forskali* revealed that proteins are the major compounds. However, fat content is rare. We note that the protein content constitutes 9.7% of the fresh *H. forskali* body wall while total fats do not exceed 0.4%. These findings are in agreement with those obtained for other fresh sea cucumber species such as *H. scabra* [26], *H. tubulosa*, *H. Polii* and *H. mammata* [27]. The different drying treatments used in this study induced a significant increase in the protein content of the *H. forskali* trepan. In this study, the highest amount of protein for the processed sea cucumber reached 52.4%. It was obtained under 70°C and 20% oven drying conditions. This result concurs with the range that is reported by Wen *et al.* [2]. These authors found that the protein content of eight common commercially processed sea cucumber species varied from 40.7 to 63.3%.

The significant increase in the protein content levels has been similarly reported for several dried fish species [28-32]. Such a result seems to be the consequence of dehydration, which tends to concentrate protein as suggested by Wu and Mao [33] in dried fish filet. Furthermore, Chukwu and Shaba [30] reported that protein nitrogen was not lost during drying. In the contrary, the protein content increased with the reduced moisture level in catfish (*Clarias gariepinus*).

In this study, the oven dried samples show highest fat content than in raw and sun dried samples. The oven dried process seems to improve the protein quality and prevents lipid oxidation as reported for the grass carp (*Ctenopharyngodon idellus*) that is dried with the microwave process [33] and the catfish processed with an electric oven [30].

The lower lipid content observed in sundried “bêche-der” could be associated with the oxidation of fat as demonstrated by Kabahenda *et al.* [34]. Similar results were recorded by Akinneye *et al.* [35] in fish *Bonga spp*, *Sardinella spp* and *Heterotis niloticus*. In fact, the lipid of traditional salted sun-dried seafood is highly susceptible to oxidation during processing and storage [36,27]. Literature has reported that sodium chloride acts as a pro-oxidant factor by enhancing the pro-oxidant effect [37]. Thus, the salted sun-dried trepangs of *H. forskali* are more prone to oxidation than the oven dried ones because of their higher exposure to light and oxygen [36].

In terms of fatty acids composition, different drying treatments induce a significant increase of the \sum SFA while leading to a strong decline of the \sum MUFA in the body wall. These observations are in discordance with those reported by Aydın *et al.* [27] for the dried *H. tubulosa*, *H. polii* and *H. mammata*. These authors reported that the drying process results in significant decrease of the \sum SFA against an increase of the \sum MUFA for *H. tubulosa* and *H. mammata* and an elevation of both \sum SFA and \sum MUFA for *H. polii*. Concerning the \sum PUFA, we have recorded that the \sum PUFA significantly decreases in *H. forskali* after the Treat.1 and treat.5 and remain insignificantly invariable for the rest of the other treatments. Our results support the findings of Aydın *et al.* [27] who have shown that the effects of the

drying process on the SFA, MUFA and PUFA depend on species, drying methods and fatty acid types.

In this study, most of the n-3PUFAs show increasing contents after drying. Same results were recorded in fillets fish processed by hot air and microwave drying [33].

We have noted that, contrary to the raw samples, proportions of the n-3PUFAs in dried body walls of *H. forskali* were higher than those of n-6 PUFA. Consequently, we have recorded an increase of the ω -3/ ω -6 ratio. The ω -3/ ω -6 ratio is a peculiar indicator of the nutritional value of seafood [38, 39]. It is also considered as an important index of the fatty acid role in human health [40]. According to the same author, the appropriate balance recommended of the ω -3/ ω -6 ratio varies from 1.1 to 1.4. Our study indicates that the dried body walls of *H. forskali* offer an interesting ω -3/ ω -6 ratio that exceeds the recommended value to reach 5.8 under the oven Treat.5. Hence, *H. forskali* trepan could be considered as an important food additive that can contribute to an equilibrated polyunsaturated fatty acid intake.

Among the n-3PUFA, the highest mean values of the EPA and the DHA were obtained by the Treat.2. It has been reported that drying processes significantly increase the relative contents of EPA and DHA in fish [33].

Taking into consideration the drying time and the nutritional quality (total proteins and \sum PUFA) of the final product, Treat.4 (T=60°C, RH=20%) seems to offer the optimum drying conditions among the tested combinations of temperature and humidity.

ABBREVIATION

BHT	=	Butylated Hydroxytoluene
SFA	=	Saturated Fatty Acids
MUFA	=	Monounsaturated Fatty Acids
PUFA	=	Polyunsaturated Fatty Acids
DHA	=	Docosahexaenoic Acid (C22:6n-3)
EPA	=	Eicosapentaenoic Acid (C20:5n-3)
ARA	=	Arachidonic Acid (C20:4n-6)

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest

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