Profile of Nutrition and Hazards of Om-Elkholool (*Donax Trunculus)* and Gandofly (*Ruditapes Decussatus*) Clams From Alexandria, Egypt

Sherief Mohammed Sayed Abd-Allah

Assistant Professor, Department of Food Hygiene "Meat Hygiene", Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt

Email:sherief74@yahoo.com

ABSTRACT

Clams are delicate nutritious food; however they can harbor potential health hazards. The current work aimed to investigate and compare some of the nutritive criteria and hazards of Om-Elkholool (*Donax trunculus*) and Gandofly (*Ruditapes decussatus*) clams sold at Alexandria, Egypt. A total of 46 samples (22 of Om-Elkholool and 24 of Gandofly) were randomly collected from fish retailers during summer of 2017. Samples were analyzed for proximate composition (dry matter, moisture, protein, fat, and ash %). The carbohydrates and energy content was calculated. The count of coliforms, fecal coliforms, *E. coli* and *Cl. perfringenes* (MPN/g) was determined. Concentration (mg/kg) of lead and cadmium in 10 randomly selected samples of each type were estimated. The dry matter, moisture, protein, fat, ash and carbohydrates percentages mean values for Om-Elkholool "Om" samples were 30.37±0.22, 69.60±0.21, 8.49±0.14, 1.29±0.03, 18.63±0.09, and 1.99±11, respectively, while for Gandofly "Gd" samples were 16.81±0.21, 83.28±0.2, 8.69±0.13, 1.22±0.03, 3.43±0.09, and 3.37±10, respectively. The gross energy content (Kcal/100g) mean value was 53.55±0.88 for Om and 59.24±0.85 for Gd. Coliforms, fecal coliforms and *E. coli* (MPN/g) could not be counted in any of the samples of Om, while were counted in 75.0, 4.17, and 4.17% of Gd samples, respectively. *Cl. perfringenes* (MPN/g) was counted in 22.73 and 12.5% of Om and Gd samples, respectivly. Lead concentrations mean value (mg/kg) was 5.38±0.86 and 9.2±0.78, while cadmium mean value was 0.88±0.08 and 0.57±0.04, in Om and Gd samples, respectively. Lead concentrations exceeded the permissible limit in all the analyzed samples (100%) of Om and Gd, while cadmium concentrations exceeded the limit in 40 and 0.0% of the samples, respectively. The mean values of some studied items (dry matter, moisture, ash, carbohydrates, energy, lead, and cadmium) were significantly differ ($P<0.05$), while of some others (protein and fat) showed no significant difference ($P>0.05$). In conclusion, Om and Gd from Alexandria are nutritious with appreciable protein and ash content. They seem nearly parallel in nutritive value. However, they could pose a serious risk for human health (harbor potential bacterial pathogens, and toxic heavy metals) especially with respect to Gd. It is advised to properly cook rather than the habit of consuming them raw; and to be consumed in not large quantities and on sporadic rather than on regular bases.

Key words: *Clams, Om-Elkholool (Donax trunculus), Gandofly (Ruditapes decussatus), Proximate Composition, Bacterial, Lead, Cadmium.*

1. INTRODUCTION

Molluscan bivalves (shellfish with two shell valves) are a popular and nutritious food caught and farmed widely with dramatic increase in their consumption worldwide (Obirikorang et al., 2013). They constitute a very important fisher resource globally, with the principal commercial species (oysters, mussels, scallops and clams) amounting to 1.8 million tonnes from marine and freshwater capture fisheries and 12.8 million tonnes from aquaculture. Clams, the most successful species used for human consumption, account for more than 38% of the global bivalves production and in terms of economic value represent the second most important group next to scallops (FAO, 2013).

Molluscan bivalves are highly appreciated as an excellent source of highly digestible protein, provide all the essential amino acids, bioactive peptides, and essential fatty acids for maintenance and growth of the human body; being also rich in polysaccharides, and essential vitamins (Friedman, 1996; Caglak et al., 2008; Fernández et al., 2015; Hu et al., 2018). As well, they have been presented as important source of macro "Ca, K, Mg, Na, P and S" and microelements "Fe, Zn, Cu and Mn" (Rittenschober et al., 2013). They are considered suitable as cardioprotective diets due to their low cholesterol content and low atherogenic and thrombogenic indices (Karnjanapratum et al., 2013; Anacleto et al., 2014a). They evidence balanced ratios of PUFA/SFA and n.3/n.6PUFA (HMSO, 1994).

In recent the consumer becomes more aware about the nutritive benefits of the food he consumes. For that information on the proximate composition is of importance. Bivalves edible tissues are mainly composed by moderate protein (9.17%) and high carbohydrates "glycogen" contents (2.6-7.0%), but low crude lipid (0.8-2.3%), cholesterol (28-85 mg/100 g) and energetic values (58-105 Kcal/100 g). They have ash content up to 2.0% (Silva and Batista, 2008; Karnjanapratum et al., 2013). Bivalve molluscs nutritional characteristics influenced by habitats, season, feed, species, as well as gametogenesis and spawning cycle (Berge and Barnathan, 2005; Li et al., 2010).

Bivalves are characteristically tender and easily digested, which make them attractive to the consumers. They usually consumed raw or lightly cooked. The consumer interest in them makes significant contribution to food security, which requires that seafood products beside provide nutritional benefits, should posing minimal health hazard (Serfor-Armah et al., 2010; Obirikorang et al., 2013). In the interest of consumer safety it is important to evaluate the hazards associated with bivalve consumption.

In seawater, bivalves may be exposed to different contaminants such as bacteria, viruses, parasites, biotoxins, heavy metals, pesticides, or drug residues (Huss et al., 2003; Topic Popovic et al., 2010). Because bivalves are filter feeders, pass enormous volumes of water across their gills to obtain oxygen and food, they can accumulate human pathogens especially from waters with sewage pollution; usually at higher concentrations several folds higher than the surrounding. Such pathogens are important with respect to the safety of bivalves especially with the habit of consuming them raw or lightly cooked which increases the pathogen-associated risk (WHO, 2010; Khora, 2014). Bacterial pathogens found in shellfish and involved in foodborne diseases in humans include those naturally occurring (e.g. *Vibrio* sp.), those associated with faecal contamination (e.g. *Clostridium* sp. and *E. coli*), and those from cross-contamination during food preparation and processing (e.g. *Clostridium perfringens*) (ICMSF, 2010; Oliveira et al., 2011).

Chemical pollution in shellfish-growing waters is a worldwide problem in coastal areas (Almeida and Soares, 2012). Consumers in recent surveys were found to be are more concerned about chemical contaminants in foods, in comparison with microbial hazards, because of their low metabolization, and ability to bioaccumulate with long-term adverse effects (Censi et al., 2006; Kher et al., 2013). Because of its filtration nature, bivalves growing in waters polluted with industrial and agricultural chemicals can concentrate toxic elements (e.g. lead and Cadmium) in their tissues, often several folds higher than in the sediment where they are buried. Consumption of shellfish so contaminated presents an additional public health problem (Karouna-Renier et al., 2007; Figueira et al., 2011). Lead can cause developmental neurotoxicity in children and cardiovascular effects in adults, while cadmium can cause kidney damage (EFSA 2010; Khora, 2014).

Within bivalve molluscs, clams are the most common species used for consumption in Mediterranean countries. *Donax trunculus* "wedge shell" and *Ruditapes decussatus* "grooved carpet shell" are two important clam species extensively harvested from natural beds in Egypt, with high densities in the shallow inshore waters. *Donax trunculus* forms extensive, dense beds in exposed sandy beaches; and *Ruditapes decussatus* lives in muddy-sand sediments of tidal flats or shallow coastal areas (Gabr Howaida, 1991; Kandeel, 1992; FAO, 2012). They are commonly consumed in Alexandria and somewhere else in Egypt, especially during summer, being highly appreciated by local consumers for their delicacy; somewhat rich in protein of high biological value, beside many other nutrients offer a variety of health benefits to the consumer. As well, they require minimal processing when consumed raw or lightly cooked.

Despite nutritious, Om-Elkhlool and Gandofly as filter feeders can represent potential health hazards (microbial and chemical) to the populace, especially when taken from polluted waters. Besides, fishermen, wholesalers and retailers usually trade them without sanitary control. Along the trade chain, they are subjected to wide temperature variations and not maintained in refrigerated conditions, often.

The aim of the current study is to estimate proximate composition and to evaluate bacterial (coliforms, fecal coliforms, *E. coli* and *Clostridium perfringens*) and toxic chemical (lead and cadmium) contaminants in fresh Om-Elkholool (*Donax trunculus*) and Gandofly (*Ruditapes decussatus*) available for sale in Alexandria, Egypt, in a trial to ascertain their nutritious and safety with regard to public health.

2. MATERIALS AND METHODS

2.1.Collection of Samples:

A total of 46 samples of bivalve molluscs (22 of Om-Elkholool "Om" and 24 of Gandofly "Gd") were randomly collected, at the early morning during the summer of 2017, from fresh fish retailers at Alexandria city, Egypt. Samples were collected separately in sterile polyethylene bags and dispatched to the laboratory under refrigerated condition. When reached the lab samples were washed of external dirt and kept chilled in new sterile polyethylene bags. Samples not used for direct preparation were stored frozen.

2.2.Preparation of Samples:

Only samples with closed shell were used for the analysis. The bivalve shell was opened using sterile scalpel and the content was collected and thoroughly homogenized in sterile mortar under aseptic condition.

2.3.Estimation of Proximate Composition (AOAC, 2000):

Of the prepared sample, 20g was used for estimation of moisture percentage. For protein 0.5g of the dried sample was used in Kjeldahl method with nitrogen to protein conversion factor of 6.25. Fat was estimated in 1g of the dried sample using Soxhlet method with slight modification (Abd-Allah and Ismail, 2016). The ash percentage was estimated in 1g of the dried sample in muffle furnace at 600ºC till complete incineration (white ash formed) "~4 - 6hrs".

NB: All estimations on the dry weight were converted according to the equation of Jurgens and Bregendahl (2007) into the wet weight basis:

$$\text{Nutrient wet basis\%} = \frac{\text{nutrient dry basis\% X dry matter \%}}{100}$$

The dry matter and the carbohydrates percentages were calculated by difference:

Dry matter % = 100 – moisture %

Carbohydrates % = 100 – (moisture % + protein % + fat % + ash %)

2.4.Energy Content

2.4.1. The gross energy content (kcal/100g) of the sample

Calculated according to the equation of Merrill and Watt (1973):

Gross energy value (Kcal/100g) = (protein% x 4) + (fat% x 9) + (carbohydrates% x 4).

2.4.2. Energy derived from each of protein, fat or carbohydrates per 100g sample

Energy from protein= protein% X 4

Energy from fat= fat% X 9

Energy from carbohydrates = carbohydrates% X 4

2.5.Bacteriological Analysis:

2.5.1. Preparation of dilutions

Ten grams of the prepared sample was weighed under aseptic condition into sterile polyethylene bag and homogenized with 90 ml of sterile 0.1% peptone water in stomacher (Seward 400) for 2 min. Tenfold serial dilution (10^{-2} then 10^{-3}) was further prepared in test tubes each containing 9 ml of sterile 0.1% peptone water.

2.5.2. Coliforms, fecal coliforms and E. coli counts (MPN/g)(AOAC, 1980)

Most probable number (MPN) using three tubes dilution method was applied for the count.

2.5.2.1. Coliforms count (MPN/g)

Of the dilutions 10^{-1}, 10^{-2}, and 10^{-3}, lauryl sulphate broth was inoculated for presumptive count, and for confirmatory count brilliant green bile (2%) broth was used as inoculation medium. Inoculated broth was incubated at 35±0.5ºC for 24 - 48hrs for each step.

2.5.2.2. Fecal coliforms count (MPN/g):

The EC medium was used for the inoculation, incubated at 45±0.5°C in thermostatically controlled water bath for 48hrs.

2.5.2.3. E. coli count (MPN/g)

The Levine Eosin Methylene Blue "EMB" agar was used for inoculation. Plates were incubated at 35±0.5ºC for up to 48hrs.Typical colonies were purple with dark center (nucleated) and with green metallic sheen.

2.5.3. Clostridium perfringens count (MPN/g) (Beerens et al., 1980)

Of the tenfold dilutions (10^{-1}, 10^{-2}, and 10^{-3}), lactose sulfite broth was inoculated. Media was incubated at 46±0.5°C for up to 48hrs. Positive tubes showed blackening with gas collection in the Durham tubes'.

2.6.Estimation of Lead and Cadmium Contents

Ten samples of each bivalve type were randomly selected for estimation of lead and cadmium contents. Of the wet prepared sample 1g was used for the estimation. Samples digestion and preparation were according to the procedure entitled by Finerty et al. (1990) with minor modification

(Abd-Allah and Ismail, 2017). The final dilution was made up to 25 ml in volumetric flask. Quantitative determination of metals concentration in the filtrate was made using "Inductively Coupled Plasma Emission Spectrometer", iCAP 6200 (Thermo Scientific, USA); [Central laboratory for chemical analysis, Faculty of Agriculture, Assiut University, Assiut, Egypt] at wavelength of 220.353 nm for lead and 214.438 nm for cadmium. The minimum detection limit for lead was 1.06 μg/L while, for cadmium was 0.07 μg/L. Results were expressed as mg/kg on wet weight basis.

2.7. *Statistics*

The obtained results were analyzed statistically using SPSS (2001) program. The data were subjected to ANOVA analysis and represented as mean ± standard error (mean±SE). The difference between means was calculated based on Duncan's multiple range test at significance level of P<0.05.

3. RESULTS

The given results in Table 1 showed the proximate composition (%) of Om-Elkholool (*Donax trunculus*) and Gandofly (*Ruditapes decussatus*) analyzed samples. The dry matter content of Om-Elkholool "Om" samples were in the range of 28.98 - 31.27 with a mean value of 30.37±0.22, while of Gandofly "Gd" samples ranged from 14.78 to 18.98 with a mean of 16.81±0.21. The moisture, protein, fat, ash and carbohydrates contents of Om samples were in the range of 68.73 – 71.02, 7.99 – 9.15, 1.06 – 1.49, 17.66 – 19.44 and 1.04 – 2.80, with means value of 69.60±0.21, 8.49±0.14, 1.29±0.03, 18.63±0.09 and 1.99±11, respectively. For Gd samples, the previous items were in the range of 81.02 – 85.22, 6.74 – 10.37, 0.90 – 1.60, 3.15 – 3.92 and 1.59 – 4.22, with means value of 83.28±0.20, 8.69±0.13, 1.22±0.03, 3.43±0.09 and 3.37±10, respectively. The dry matter and ash contents were significantly higher (P<0.05) in Om, while moisture and carbohydrates were higher (P<0.05) in Gd samples. Protein and fat contents were not significantly differ (P>0.05) between Om and Gd samples (Table 1). The gross energy (Kcal/100g) content showed min, max and mean values of 50.84, 57.41, and 53.55±0.88 for Om; and 49.19, 70.24, and 59.24±0.85 for Gd. The gross energy mean values were significantly higher (P<0.05) in Gd samples. The min, max and mean values (Kcal/100gm sample) of the energy derived from fat content were 9.5, 13.39, and 11.63±0.30; from protein content were 31.97, 36.61, and 33.96±0.54; and from carbohydrates content were 4.16, 11.22, and 7.96±0.42, respectively for Om; while in case of Gd, the min, max, and mean values for energy from fat content were 8.12, 14.39, and 10.98±0.29; from protein content were 26.96, 41.50, and 34.77±0.52; and from carbohydrates content were 6.38, 16.86, and 13.48±0.41, respectively (Table 2)

Table 1: Statistical results for proximate composition (%) "wet weight" of Om and Gd samples

Parameter	Om (n=22)			Gd (n=24)		
	Min	Max	Mean ± SE	Min	Max	Mean ± SE
Dry Matter	28.98	31.27	30.37 ± 0.22^{a}	14.78	18.98	16.81 ± 0.21^{b}
Moisture	68.73	71.02	69.60 ± 0.21^{b}	81.02	85.22	83.28 ± 0.20^{a}
Protein	7.99	9.15	8.49 ± 0.14^{a}	6.74	10.37	8.69 ± 0.13^{a}
Fat	1.06	1.49	1.29 ± 0.03^{a}	0.90	1.60	1.22 ± 0.03^{a}
Ash	17.66	19.44	18.63 ± 0.09^{a}	3.15	3.92	3.43 ± 0.09^{b}
Carbohydrates	1.04	2.80	1.99 ± 11^{b}	1.59	4.22	3.37 ± 10^{a}

Min= minimum Max= maximum Mean ± SE= mean ± standard error
In the same row means with different superscripts are significantly differ (P<0.05)

Table 2: Statistical results for energy content (Kcal/100g soft tisssue) of Om and Gd samples

Parameter	Om (n=22)			Gd (n=24)		
	Min	Max	Mean ± SE	Min	Max	Mean ± SE
Gross Energy	50.84	57.41	53.55±0.88[b]	49.19	70.24	59.24±0.85[a]
E Fat[1]	9.5	13.39	11.63±0.30[a]	8.12	14.39	10.98±0.29[a]
E Ptn[2]	31.97	36.61	33.96±0.54[a]	26.96	41.50	34.77±0.52[a]
E Cab[3]	4.16	11.22	7.96±0.42[b]	6.38	16.86	13.48±0.41[a]
E Fat %[4]	18.49	24.86	21.72±0.39[a]	14.69	22.04	18.49±0.38[b]
E Ptn %[5]	59.57	70.17	63.44±0.53[a]	58.81	67.48	58.74±0.50[b]
E Cab %[6]	8.11	20.61	14.84±0.64[b]	10.97	28.69	22.77±0.65[a]

Min= minimum Max= maximum Mean ± SE= mean ± standard error

[1, 2, 3] Energy derived from fat, protein, and carbohydrates, respectively.

[4, 5, 6] Percentage of energy derived from fat, protein, and carbohydrates, respectively.

In the same row means with different superscripts are significantly differ ($P<0.05$)

Table 3: Statistical results for bacterial quality parameters (MPN/g) of Om samples (n=22)

Parameter	Positive samples		Min	Max	Median
	No.	%			
Coliforms count[1]	0	0.0	-	-	-
Fecal coliforms count	0	0.0	-	-	-
E. coli count	0	0.0	-	-	-
Cl. Perfringenes count (MPN/g)	5	22.73	3.6	7.3	3.6

Min= minimum Max= maximum [1]The confirmatory count

Table 4: Statistical results for bacterial quality parameters (MPN/g) of Gd samples (n=24)

Parameter	Positive samples		Min	Max	Median
	No.	%			
Coliforms count[1]	18	75.0	3	93	9.1
Fecal coliforms count	1	4.17	3.6	-	-
E. coli count	1	4.17	3.6	-	-
Cl. Perfringenes count[2]	3	12.5	3.6	-	-

Min= minimum Max= maximum [1]The confirmatory count

[2]The 3 positive samples showed the same count (3.6 MPN/g for each)

Table 5: Statistical results for lead and cadmium concentrations (mg/kg) in Om and Gd samples

Parameter	Om (n=10)			Gd (n=10)		
	Min	Max	Mean ± SE	Min	Max	Mean ± SE
Lead	3	10.5	5.38±0.86[b]	5.25	12.75	9.2±0.78[a]
Cadmium	0.525	1.1	0.88±0.08[a]	0.375	0.75	0.57±0.04[b]

Min= minimum Max= maximum Mean ± SE= mean ± standard error

In the same row means with different superscripts are significantly different ($P<0.05$)

Table 6: Acceptability (%) of Om and Gd samples based on their lead content (mg/kg)

	Samples tested	Permissible limits (mg/kg) [1]	Samples within limits (%)	Samples exceeded limits (%)
Om	10	1.5	0 (0.0%)	10 (100%)
Gd	10		0 (0.0%)	10 (100%)

[1]Permissible limit according to the Egyptian standards (ES, 2010).

Table 7: Acceptability (%) of Om and Gd samples based on their cadmium content (mg/kg)

	Samples tested	Permissible limits (mg/kg) [1]	Samples within limits (%)	Samples exceeded limits (%)
Om	10	1	6 (60%)	4 (40%)
Gd	10		10 (100%)	0 (0.0%)

[1] Permissible limit according to the Egyptian standards (ES, 2010).

The percentage of energy derived from fat, protein and carbohydrates contents were ranged from 18.49 to 24.86, 59.57 to 70.17 and 8.11 to 20.61, with means of 21.72±0.391, 63.44±0.53, and 14.84±0.64, respectively in Om; and from 14.69 to 22.04, 58.81 to 67.48 and 10.97 to 28.69, with means of 18.49±0.38, 58.74±0.50, and 22.77±0.65, respectively, in Gd as declared in Table 2. The percentage of energy derived from fat and protein were significantly higher ($P<0.05$) for Om, while that from carbohydrates was higher ($P<0.05$) for Gd samples.

Coliforms, fecal coliforms and *E. coli* showed no detectable count (MPN/g) in any of Om samples, while *Cl. perfringenes* (MPN/g) was counted in only 5 (22.73%) samples with range of 3.6 – 7.3 and median value of 3.6 (Table 3). For Gd, coliforms could be count (MPN/g) in 18 (75.0%) of the 24 analyzed samples with range of 3 to 93 and median value of 9.1, while fecal coliforms and *E. coli* showed count each in only one sample with MPN value of 3.6/g. *Cl. perfringenes* showed count in 3 samples of Gd with MPN value of 3.6/g for each (Table 4).

The data in Table 5 showed that Pb concentrations (mg/kg) in Om samples were in the range of 3 – 10.5 with a mean of 5.38±0.82, while in Gd ranged from 5.25 to 12.75 with a mean of 9.2±0.82. The concentrations of cadmium ranged from 0.525 to 1.1 and 0.375 to 0.75, with mean value of 0.88±0.06 and 0.57±0.06 in Om and Gd samples, respectively. Lead concentrations mean value was significantly higher ($p<0.05$) in Gd samples, while cadmium mean value was higher ($P<0.05$) in Om samples. All the analyzed samples (100%) of Om and Gd showed lead concentrations exceeded the permissible limit (1.5 mg/kg) set by the Egyptian Organization for Standardization (Table 6). On the other hand, 40% of Om samples showed cadmium concentrations exceeded the allowed limit (1 mg/kg), while none of the Gd samples (0.0%) showed concentration above the limit (Table 7).

4. DISCUSSION

In the last decade, the importance of clam bivalves has been increasing in terms of landing volumes and economic value among other marine resources. Their consumption as relatively cheap and good source of animal proteins has been dramatically increasing worldwide. They have been promoted as healthy food in many countries, with an exceptional nutritional value making them ideal for the human diet (Serfor-Armah et al., 2010; Ngo et al., 2012). Within clams, *Donax trunculus* (Om-Elkholool) and *Ruditapes decussatus* (Gandofly) are important species consumed in Egypt (Pinello et al., 2020).

4.1. Basic Composition

The proximate compositions of bivalve molluscs varied among species and even between individuals of same species, being affected by sex, maturation stage, harvest area, catching season, beside feed composition and availability (Orban et al., 2002; Chi et al., 2007; Karnjanapratum et al., 2013).

The results of the proximate composition "%" (dry matter, moisture, protein, fat, ash, and carbohydrate) of the soft tissue of Om (*Donax trunculus*) and Gd (*Ruditapes decussatus*) samples are presented in Table 1. The dry matter content was higher ($P<0.05$) in Om than in Gd with mean values "%" of 30.37±0.22 and 16.81±0.21, and range of 28.98 to 31.27 and 14.78 to 18.98, respectively. The moisture content “%”, however, was higher ($P<0.05$) in Gd with mean values of 83.28±0.203 and 69.60±0.212, and range of 81.02 to 85.22 and 68.73 to 71.02 in Gd and Om, respectively. These results were within the values found by Mohammad Samya (2015) for *Donax trunculus* (moisture 56.2 – 76.78) and *Donax semistriatus* (moisture 54.94 – 83.31) from Suez Canal and Idku in Egypt. Bejaoui et al. (2018) in Tunisia detected nearly similar dry matter and moisture content (15% and 85%, respectively) for *Venerupis decussata*. In some other studies, varying percentages of dry matter / moisture content were recorded for a variety of bivalve species including *Galatea paradoxa* “20.6 / 79.4” by Serfor-Armah et al. (2010) in Hungary, *Callista chione* “19.45 / 80.55” by Papaioannou et al. (2016) in Greece, *Anadara granosa* “18.6 / 81.4” and *Meretrix casta* “28.5 / 71.5” by Venugopal and Gopakumar (2017), and *Crassostrea gigas* “20.08 / 79.92” by Yijing et al. (2018) in china, respectively. Moisture content is one of the most important biochemical constituents of the shellfishes, having functional effect on some quality characteristics and affects the microbiological stability of the food product as well (El-Lahamy et al., 2018; Pinello et al., 2020).

The protein content of Om and Gd was nearly similar with mean value (%) of 8.49±0.14 and 8.69±0.13, and range of 7.99 - 9.15 and 6.74 - 10.37, respectively. Anacleto (2014) in Portugal estimated higher content of protein in *Ruditapes* spp. “11.7%”, but nearly similar content in *Crassostrea* spp. “9.4%”. Higher protein contents (%) were also recorded by Serfor-Armah et al. (2010) for *Galatea paradoxa* “15.3±0.2”; Venugopal and Gopakumar (2017) for mixed oyster “11.4”, *Anadara granosa* “14.9” and *Meretrix casta* “15.7”; and Yijing et al. (2018) for *Crassostrea gigas* “10.59±0.18”. Clams protein is of high quality with PER value (protein efficiency ratio) similar to that of milk and eggs. It provides all the essential amino acids for maintenance and growth of the human body (Friedman, 1996; Venugopal, 2006). According to US Dietary Guidelines (2015), which recommended daily intake of 56 g protein by males aged 19 to 30; 100g of raw Om or Gd (containing 8.5g protein) will fulfill 15% of that daily protein requirement.

The fat content (%) of Om “1.29±0.034” was close to that of Gd “1.22±0.032”. Total lipid content around 1.0% was recorded in *Ruditapes decussatus* by Gonçalves et al. (2009) which seems close to the current result. Anacleto (2014) recorded slightly lower fat content in *Ruditapes* spp. “0.9%” and higher content in *Crassostrea* spp. “2.3%”. Bejaoui et al. (2018) also estimated somewhat higher mean value of fat in bivalve *Venerupis decussata* “2.71±0.6”. Serfor-Armah et al. (2010), Papaioannou et al. (2016), Venugopal and Gopakumar (2017), and Yijing et al. (2018) reported different fat content in a variety of bivalve species. Bivalves lipids are known to be low in saturated fatty acids and relatively rich in omega-3-polyunsaturated fatty acids in particular eicosapentaenoic and docosahexaenoic acids, which play a major role in the prevention and recovery from coronary heart diseases, asthma, diabetes, cancer and foetal malformations and in improving the response to inflammatory diseases, like eczema, psoriasis and rheumatoid arthritis (USADA-ARS, 2005; kristensen et al., 2016). They evidence balanced ratios of PUFA/SFA and n.3PUFA/n.6PUFA, as the minimum recommend values“0.45 and 0.25, respectively” are exceeded for most species (HMSO, 1994).

The ash content (%) mean value of Om samples (18.63±0.091) was greatly higher ($P<0.05$) than that of Gd “3.43±0.087”. It ranged from 17.66 to 19.44 in Om and from 3.15 – 3.92 in Gd. Lower ash contents were reported by Serfor-Armah et al. (2010) “1.4±0.13”, Venugopal and Gopakumar (2017) “up to 2%”, and Yijing et al. (2018) “2.15±0.48”, in different species of clams including *Galatea paradoxa*, *Anadara granosa* and *Meretrix casta*, and *Crassostrea gigas*, respectively. Silva and Batista (2008) mentioned that the ash content varies according to several biological and environmental parameters, including bivalve species (0.8-3.0%) and origin. Tendency to accumulate sand in mantles and gills is a character and one of the problems specific to calm species (Ward and Hackeny, 1991; Pinello et al., 2020), which might be related to the very high ash content obtained in the current study for the Om samples. Wet storage is usually used to de-sand those bivalves before marketing. Like other bivalve molluscs, Om and Gd could be considered as a good source of nutritionally important minerals contain almost all essential elements in their edible parts.

Bivalve molluscan shellfish characterized by having a larger quantity of carbohydrate, mainly stored as glycogen in their flesh. High content of glycogen usually reflected in the quality and taste of bivalves (Oliveira et al., 2006). The obtained carbohydrate content of Gd was significantly higher ($P<0.05$) than of Om, with mean values of 3.37±103 and 1.99±108, respectively. Lower value of carbohydrates was found by Anacleto (2014) in *Ruditapes* spp. "2.6%", while somewhat higher value was estimated in *Crassostrea* spp. "4.9%". Higher glycogen contents were also recorded by Serfor-Armah et al. (2010) in *Galatea paradoxa* "22.1±0.2"; and by Karnjanapratum et al. (2013) in Asian hard clam "7.9%"; while slightly higher content was estimated in *Crassostrea gigas* "4.41±0.23" by Yijing et al. (2018). High glycogen levels in bivalves may be interpreted as a metabolic adaptive strategy to endure environmental changes. Under stressful conditions glycogen is primarily used for the maintenance of animal condition (Patrick et al., 2006). The species difference in glycogen content might be related to variation in the size and soft tissue content. Levels of glycogen being also vary depending on time within the spawning cycle, reaching its lower peak immediately after spawning (Matias et al., 2013).

4.2. Energy Content

The gross energy content (Kcal/100g) of Om and Gd was low in general (Table 2), however it was found to be significantly lower ($p<0.05$) in Om than in Gd edible tissue. The mean value of energy content was 53.55±0.88 and 59.24±0.85 for Om and Gd, respectively. This seems close to that obtained by Anacleto (2014) for *Ruditapes* spp. "58 Kcal/100g", but lower than that obtained for *Crassostrea* spp. "81 Kcal/100g". Serfor-Armah et al. (2010) also recorded higher energy content "85.8±0.2" for *Galatea paradoxa* bivalve. Dong (2001) declared that bivalves are low in calories due to their low lipid contents which agreed with the current findings. The present energy values showed that the highest percentage of energy from edible tissue was attributed to the protein content "63.44% in Om, and 58.74% in Gd", while the lowest percentage was attributed to the carbohydrates content; which agree with Vladau et al. (2008), found that protein accounts for 80 to 90% of the energy content of the fish.

4.3. *Bacterial*

Despite their nutritional value, however, as filter-feeders inhabiting estuaries constantly subjected to contamination and climate variability, bivalves including clams concentrate contaminants (microbial and chemical) in their tissues to a much higher level than those of the surrounding sea (Lees, 2000; Fang et al., 2003). Serious safety concerns connected with the consumption of bivalves due to the presence of biological and toxicological hazards is well established (RASFF, 2012). Compared to fish, bivalve molluscs are responsible for almost the double of patients, despite being responsible for a much lower number of outbreaks (Olsen et al., 2000; Huss et al., 2003).

With consumer's risky behavior of eating them raw or lightly cooked, bivalves may then become a vector for pathogens transmission. This culinary tradition makes consumers more exigent about the bivalve quality (Lee et al., 2008). Sewage discharges in harvesting areas are the cause of bivalve contamination by a variety of microorganisms. Sewage outfall, recreational ports and storm water runoff constitute the important sources of faecal contamination (Touron et al., 2007; Roldan et al., 2011). Coliforms, fecal coliforms, and *E. coli* are used as indicator of fecal pollution. The detection of coliforms of feacal origin and *E. coli* gives relevant information regarding the food safety and sanitary conditions of clams. The high coliforms count and the presence of *E. coli* serves as an indicator for pathogenic organisms (Ward and Hackney, 1991; Silva Ana et al., 2003).

In the present work, coliforms, fecal coliforms and *E. coli* showed higher count and incidence in Gd samples. None of the mentioned bacteria could be counted in any of Om samples (<3 MPN/g). In Gd samples coliforms showed an incidence rate of 75% with count in the range of 3 to 93 and median value of 9.1 MPN/g. Fecal coliforms and *E. coli* could be counted in only one Gd sample with a count of 3.6 MPN/g and an incidence rate of 4.17% for each. *Escherichia coli* higher incidence “22%” was recorded in *Ergeria radiate* by Bassey et al (2014), while similar incidence “4.2%” was recorded in *Galatea paradoxa* by Udoh et al. (2017).

Higher count of fecal coliforms of 25 – 160 cfu/g, was found by El-Shenawy Nahla (2004), in *Ruditapes decussatus* from Ismilia, Egypt. Microbiological studies carried out in a different clam species than those used in this study also showed a greater microbial accumulation. El-Gamal Mona (2011) found high *E. coli* count in *Paphia undulate* from Ismailia, Egypt, of $4.1\text{x}10^4$cfu/g. Likewise, high total coliforms and fecal coliforms count, of $1\text{x}10^4$ and $5\text{x}10^2$ cfu/g, were found by Silva Neta et al. (2015) for *Crassostrea rhizophorae* in Brasil; and of $2.7\text{x}10^2$ and $1.8\text{x}10^2$ cfu/g by Udoh et al. (2017) for *Galatea paradoxa* in Nigeria. As well, high coliforms and *E coli* count was recorded by Efiuvwevwere and Amadi (2015) for *Crassostrea gasar* in Nigeria at both rain ($1.6\text{x}10^2$ and $1.1\text{x}10^2$ cfu/g) and dry ($1.42\text{x}10^4$ and $2\text{x}10^2$ cfu/g) seasons, respectively. Low count of coliforms was found by Bassey et al (2014) for *Ergeria radiate* in Nigeria, of 4-10 cfu/100g. In Brazil, Pereira et al. (2007) showed that oysters from Maragogipe Bay served in restaurants contained 22 to 13,000 MPN/g of total coliforms and 1.8 to 4,600 MPN/g of thermotolerant coliforms.

Total coliforms may be directly influenced by many anthropogenic activities. Several industries and farming explorations, combined with a highly populated area, contribute to the production of heavily polluted wastewaters nearby seashores. As well, in rough weather events, faecal coliforms in sediment can be released to the water column leading to increased coliform levels in water (Davies et al., 1995; Adjei-Boateng et al., 2009). Moreover, run-off from rain might carry raw sewage from the surrounding area into the seawater. This might explain the higher coliform levels detected in Gd that mainly harvested from fisheries in Alexandria in comparison to Om that harvested from fisheries in other areas e.g. Edku and Rashid (Pinello et al., 2020). Following harvest, fecal coliform level in excess of 230 MPN/l00 g indicate that the shellfish may have come from an improperly classified area, been processed under unsanitary conditions, been subjected to temperature abuse during storage, and/or experienced excessive storage time (Ward and Hackney, 1991).

In the current study, coliforms *and E. coli* count exceeded the permissible limit of 300 and 230 MPN/100g, respectively; set for raw molluscs (EC, 2007) was noticed in 4.17% of the Gd samples and none (0.0%) of Om samples. *Escherichia coli* exceeded the limit was noticed in higher percentage of Manila clam (*Tapes semidecussatus*) “35.3%” and Japanese carpet shell clam (*Tapes philippinarum*) “25%” from Poland, while in the Pacific oyster (*Crassostrea gigas*) and Razor clam (*Ensis directus*) the percentage of samples exceeded the *E. coli* limit was lower “0.0%” (Pomykała et al., 2012). *Escherichia coli* are a faecal coliform bacterium belonging to the enteric bacteria group and is widely distributed in the intestines of healthy humans and mammals. It is the only biotype of the family *Enterobacteriaceae* that is exclusively from faecal origin. Its presence in bivalves and overlying waters indicates the possible presence of other pathogens. Despite, most strains of *E. coli* are not regarded as pathogenic; they can be opportunistic pathogens that cause infections in immunecompromised hosts (Rhodes and Kator, 1998; Feng et al., 2002). Consumption of bivalves contaminated by such bacteria may lead to severe and potentially fatal foodborne diseases where gastroenteritis is the most frequent clinical syndrome (Pomykała et al., 2012).

The bacterium clostridia are ubiquitous in aquatic environments being isolated from water, sediments, fish, and shellfish. The presence of *Clostridium perfringens* indicates either sewage contamination of the marine environments where spores of the microorganisms permit their persistence; and /or poor handling hygiene of the product (Huss et al, 2003). In the current study, *Cl. perfringens* showed higher incidence in Om "22.73%" than in Gd "12.5%" samples, with a low count (MPN/g) of 3.6 – 7.3 in Om and 3.6 in Gd. Pomykała et al. (2012) recorded high incidence of spore forming anaerobe bacteria in Manila clam "88.2%", Japanese carpet shell clam "87.5%", Razor clam "66.7%" and Pacific oyster "61.5%" from Poland. *Clostridium perfringens* may have also been derived from external sources during handling and as such, the clams become transient carriers of such microbes. Numbers greater than 10^6 are necessary to cause illness. However, such quantities do not reach foods by mere contamination, but accumulate as a result of multiplication of vegetative cells with improper storage condition (Bryan, 1980).

Public health concerns related to consumption of bivalves are prompted by the fact that they are frequently consumed raw and that all soft parts of the animal including the foot and viscera are consumed rather than just the muscle tissue. Seasonality and environmental parameters (e.g., rainfall, water temperature, and salinity) are the main factors influencing bacterial concentration in bivalves. As bivalves are a protein rich food, therefore serves as a suitable substrate in supporting growth of different types of bacteria. The microbial growth will encourage food spoilage and seafood poisoning. Growth of bacteria is temperature mediated; therefore, rapid and complete cooling of the stock slows microbial growth and prolongs the storage life (Ward and Hackney, 1991; Farias et al., 2010; Derolez et al., 2012).

4.4. *Lead and Cadmium*

In the past few decades the increase in disposal of domestic wastes, shipping activities, industrial effluents into inshore waters leads to dramatic rise in the contamination of the Egyptian marine environment with heavy metals. This raised the safety concern of using fish and shellfish from Egyptian fisheries for human consumption (Abdel-Moati, 1991; Mourad, 1996). The danger of heavy metals arises from the fact that they are persistent in the environment. They can be bio-magnified in the seafood chain and finally be assimilated by human marine food consumers causing health risks. They accumulate at a rate faster than the body can eliminate them causing DNA damage and cancer in some cases (Islam and Tanaka, 2004; Yi et al., 2011). From human health risk point of view, Pb and Cd are of the most toxic metals. Human exposure is mainly via food and water (Ekpo et al., 2008).

The obtained Pb levels mean value (mg/kg) in the present study, was higher ($p<0.05$) in Gd "9.2±0.78" than in Om "5.38±0.86" soft tissues, while Cd was higher ($p<0.05$) in Om. El-Shenawy Nahla (2004) declared that heavy metals concentration in the soft parts of the shellfish were found to be inversely related to size, which is in agreement with the obtained result for lead but not for cadmium. The significantly higher levels of Pb in Gd compared to Om in the current study may also probably attribute to the differences in habitat or in biological characteristics of the two species. Toxico-kinetic studies conducted by Labrot et al. (1999) have found that the rate and level of accumulation of toxic elements depend on the species and element. Lower Pb "1.12 – 1.85" and Cd "0.32 – 0.48" levels (mg/kg) were recorded by El-Serehy et al. (2012) for *Donax trunculus* from port Said , Egypt; and by Usero et al. (2005) for *Donax trunculus* from spain "0.72 and 0.04 mg/kg, respectively". On the other hand, lower Pb but higher Cd mean values were found by Gabr Howaida and Gab-Alla (2008) for *Ruditapes decussatus* “3.11±0.2 and 0.9±0.1”, and *Venerupis pullastra* “3.71±0.1 and 1.75±0.1” from Ismailia, Egypt, respectively. El-Wazzan Eman et al. (2013) estimated varying Pb and Cd levels in the range of 0.1 – 0.32 and 0.01 – 2.24, respectively, for *Tapes decussatus* from three different localities in Alexandria during winter and spring seasons. Besides, El-Wazzan Eman et al. (2014) recorded lower levels of Pb and Cd in the range of 0.1 – 6.86 and 0.1 – 0.79, respectively for stripped Venus clam “*Chamelea gallina*” from the area of Rasheed to Burulls, Egypt. In some other studies in Egypt, lower levels of Pb and Cd were found by El-Gamal Mona (2011) in the clam *Paphia undulate* “5.61 and 0.22, respectively” from Ismailia; Ahdy et al. (2007) in *Mactra* spp. and *Mytilus* spp. clams from Alexandria; and El Nemr et al. (2012) in pool of different bivalves from the area of Alexandria to Port Said. As well, low Pb content, of 3.57 ppm, was found by Ibrahim Nesreen and Abu El-Regal (2014) in *Venerupis aureus* from Timsah Lake. Çolakoğlu et al. (2010) recorded lower Pb and Cd values in stripped Venus clam from Turkey in the range of 0.18–3.24 ad 0.04–0.69, respectively; Anacleto (2014) lower values in *Ruditapes* spp. “0.1 and 0.02” and *Crassostrea* spp. “0.1 and 0.3”; and Anacleto et al. (2014b) lower values in *Ruditapes philippinarum* “0.29 and 0.17” from Portugal, respectively.

The Egyptian Organization for Standardization (ES, 2010) set permissible limit of 1.5mg/kg for Pb and 1mg/kg for Cd in soft parts of raw bivalve molluscs. The obtained Pb concentrations in the current study exceeded the set limit in the all 10 (100%) inspected samples of Om and Gd; while, Cd levels exceeded the limit in 40% of Om and in none (0%) of Gd 10 analyzed samples. Heavy metals taken in traces bind with proteins and become non-toxic, but their high concentration, above the body tolerance level, results into severe pathological conditions. Doses below the tolerance limit, though not harmful might pose health hazards when consumed in large quantities due to bio-accumulation (Abd El-Salam et al., 2013).

Lead and cadmium seem not to participate in any metabolic functions. They are among the main toxic elements found in water bodies and may accumulate in bivalve species at high concentrations that can reach several orders of magnitude above those in the environment. They may be biomagnified in the food chain to levels that cause physiological impairment in consumers (El-Gamal Mona and Sharshar, 2004; Yap et al., 2009; Zuykov et al., 2013). Lead is a metabolic poison that binds to essential enzymes and several other cellular components and inactivates them. It affects the neurological, reproductive, renal, and hematological systems. Lead toxicity can cause the headache, learning disabilities, brain damages and hearing problems. The functionality of the thyroid and the adrenal glands is also affected. Miscarriage, male infertility and neonatal mortality may be seen. Impairment of the immune system may also occur with joint pains related to gout and cardiac fibrosis (Cunningham and Saigo Barbara, 1997; Wexler and Shayne, 1998). Cadmium toxicity may give rise to renal, pulmonary, hepatic, skeletal, reproductive effects and cancer. It is primarily toxic to kidney. Cadmium toxicity to bones leads to the famous “itai-itai” disease. Cadmium may catalyze diabetes-induced effects on kidneys. Recent investigations showed that cadmium may also play a role in the development of other cancers, such as testicular, bladder and pancreatic cancers, as well as cancer of the gall bladder (Zhu et al., 2011; Solidum Judilynn et al., 2013).

Safety of this kind of seafood can be guaranteed mainly by appropriate preventive measures at various stages of harvesting, storage, distribution, and consumption. Such measures include shellfish harvesting from approved shellfish waters (European Communities, 1991), final product inspections, and hygiene control in whole food chain. As well, the consumers should be enlightened on possible health hazards associated with the consumption of raw unapproved products. Fishermen and wholesalers should be educated on the importance of depuration as a means of bivalves decontamination before sale.

Depuration by holding bivalves in excess of clean water for a couple of days was an efficient process to reduce the levels of bacterial contaminants (e,g. coliforms and *E. coli*) and toxic elements (particularly Pb) to levels considered as acceptable for human consumption (El-Gamal Mona, 2011; Codex Alimentarius, 2013; Anacleto et al., 2015). Regulations should require depuration of bivalves before commercialization for human consumption so as to meet the health standards. The current study highlights the need for the depuration process before commercialization to diminish especially the lead contamination.

5. CONCLUSION

From the foregoing, it is clear that Om and Gd bivalve mollusks from Alexandria retail fish markets could be a good source of nutrients. They are nearly parallel in their nutritive value (have nearly the same protein and fat contents), but ash content was significantly higher in Om. Despite the bacterial quality of Om was somewhat better being met the microbiological criteria for raw bivalve molluscs intended for direct consumption (found negative for total coliforms, feacal coliforms and *E. coli*), their Pb content however, was exceeding the permissible limit. As well, some samples were found containing *Clostridium perfringens* and high Cd levels. Gandofly was found to contain high levels of Pb far exceeding the limit. In addition, harbours microorganisms including those that are pathogenic (*E. coli* and *Clostridium perfringens*) in some samples. In conclusion, clams especially Gd, from this region despite nutritious; harbour in them some hazards (bacterial and chemical) that could pose risk to public health. It is to recommend proper cooking of the Om and Gd, rather than eating them raw; as a public health safeguard against pathogens. Better not to consume them in large quantities and on frequent bases to avoid the hazard effect of Pb and Cd. In addition, Clams should be depurated before marketing. They should be transported and stored hygienically under chilled conditions in order to encourage desired products and to discourage growth of bacteria of public health interest.

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