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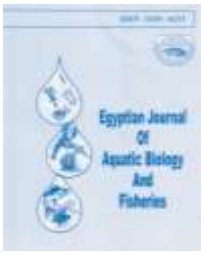

Effect of technological processing on the safety of Indian mackerel (*Rastrelliger kangurata*) from Suez, Egypt

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ABSTRACT

This study investigated the effect of different fish processing technologies on the bacteriological safety and heavy metal contents of Indian mackerel fish products. Mackerel was processed by salting (dry salt 15%), hot marinating (2.5% acetic acid and 3% NaCl) or canning (hot vegetable oil, brine 3% or tomato sauce 8%). Fresh, chilled fish from the local market had an aerobic colony count of 5.1± 0.2 logs CFU/g and the presence of *Salmonella enterica* was confirmed using API diagnostics and 16S rDNA. Marination and salting resulted in 4 and 1.3 logs CFU/g reduction in aerobic colony counts, respectively. Canning eliminated all viable bacterial growth, regardless of filling medium (vegetable oil, brine or tomato sauce). Processing technologies had no effect on the heavy metal (Hg, Cd, Pb, and Cu) content of the fish products.The estimated daily and weekly intake values of Pb and Cd from fresh fish and their products were below the recommended safety regulations. Canning, followed by marination, were the safer options for the preservation of Indian mackerel (compared to salting).

INTRODUCTION

Fish has a high nutritional value and is considered a perishable food when its quality deteriorate during storage and/or during processing. Many consumers change their acceptance and preference towards fish products over time. Pathogenic bacteria such as *Listeria monocytogenes*, *Salmonella enterica* and *Vibrio parahaemolyticus* are commonly associated with fish products (Song *et al*., 2011) and the risk associated with these pathogens needs to be established for general public health. Thus, aerobic colonies and pathogenic bacteria of public health importance are used to examine the quality of fish and its products (Mol and Tosun, 2011).

The preservation of fish and the processing options used during fish processing have a great impact on the safety and sensory attributes of the final product. Manufacturing procedures such as control of salt content, pH and heat are very important to ensure the safety of the final fish product (Oxen and Knorr, 1993; Van Opstal *et al*., 2003; Molina-Höppner *et al*., 2004; Sevenich *et al*.,2013). However, cured fish products containing different amounts of preservatives (salt or vinegar) and thermally-processed products have different safety levels.

Increasing human activities near coastal areas have caused more attention regarding risks to seafood contamination and thus human health resulting from environmental pollution (Shreadah *et al*., 2006; Said *et al*. 2006; Younis & Nafea 2012; El Zokm *et al*., 2015; Younis, 2018a; Younis, 2018b).

Heavy metal elements such as mercury (Hg), lead (Pb) and cadmium (Cd) are the most common contaminant heavy metals in the aquatic environment and their accumulation in fish leads to potential health risks upon consumption (Kalay *et al*., 1999; Emami *et al*., 2005; Younis *et al*., 2014; Soliman *et al*., 2018). Continuous evaluations and safety guidelines have been provided by the Food and Agriculture Organisation of the United Nations (FAO) and World Health Organisation (WHO) (FAO/WHO, 2004) to support efforts to control this risk.

Total Egyptian fish landings from the Red Sea and Gulf of Suez have increased from 14,700 tons in 1980 to around 45,053 tons in 2014 (FAO, 2004; FSYB, 2014). The Indian mackerel fish (*Rastrelliger kanagurta*) is a pelagic species regarded as an important source of inexpensive protein and comprises about 22% of all catch from Egyptian waters. Due to the importance and widespread availability of salted, marinated and canned fish products in Egypt as well as many other African, Asian and European countries, the present investigation aimed to study the effect of these processing technologies on the heavy metal content and bacteriological safety of salted, marinated and canned Indian mackerel products.

MATERIALS AND METHODS

Materials

Fresh marine Indian mackerel (*Rastrelliger kangurata*), known locally as Cascomry, with an average length of 23± 3.0 cm and weight of 135± 19.7g were used in this study. The fish were purchased from ten different local fish markets (2 kg fish from each store) in Suez, Egypt in March 2016. A total of 20 kg of fish were transferred to the laboratory in polystyrene boxes filled with crushed ice. Ice was prepared in the lab using sterilised distilled water and was hygienically prepared and handled.

Other materials included refined coarse salt, obtained from the El-Nasr Salines Company, natural sugarcane vinegar (5%) from the Agrocorp Company (Suez, Egypt), tomato sauce (22%) from Heinz Egypt, edible blend oil (sunflower oil and soybean oil) from the Food Industry Holding Company (FIHC) and glass jars (capacity 250g) with screw metal lids and polyethylene bags were obtained from the Suez commercial market (Egypt).

Technological processes

The fresh Cascomry were washed and divided into four groups. One group was used fresh and the other three were assigned to one of three treatments: dry salted, hot marinated or canned products, as follows:

Salting process

Fish were placed in layers interposed with layers of salt (15%) in tight polyethylene plastic containers. The packs were stored at room temperature for two weeks until ripening.

Hot marinating process

Fish were filleted, quickly washed under running water and soaked in 10% brine solution for 10 min. The salted fillets were steamed under atmospheric pressure for 15 min, cooled at room temperature (23°C± 2°C), packed in glass jars and covered at 1:1 (w/v) ratio with marinating solution (2.5% acetic acid and 3% sodium chloride, NaCl). The final product was prepared to be a fast-processed product, stored in a refrigerator at a temperature of 4 ± 1°C for only 3 days.

Canning process

Washed fish were de-headed, eviscerated, trimmed of fins and cut into steaks (3cm long) then quickly washed under running water. The steaks were immersed in 10% brine solution for 10 min, and then steamed at atmospheric pressure for 15 min. The steamed steaks were packed into glass jars, then hot oil, brine (3%) or sauce (8% Total soluble solids) was added before jars were exhausted and lids sealed immediately. The closed glass jars were transferred into a vertical retort and treated under pressure (15 lb/in^2) for 60 min. After the heat processing step, the jars were cooled immediately and stored at room temperature for 7 days.

Analysis

Fish fillets were prepared from fresh fish (20 samples), salted fish (20 samples), with each sample representing one single fish. Marinated fish fillets (20 samples) were selected randomly from different jars. Canned fish steaks (7 samples) were selected from each of the filling media (vegetable oil, brine or tomato sauce) to represent 21 total canned fish samples. Samples were then prepared for the following assessments.

pH measurement

Fish samples were blended with distilled water at a ratio of 1:9 (W/V). The pH of the solution was determined by a calibrated pH meter (PH700 Benchtop, Apera Instruments, LLC) according to AOAC (1995).

Bacterial detection and enumeration

For bacterial counts, samples (25 g each) were diluted in 225 ml 0.1% sterilised peptone water (Lab M, Lancashire, UK) and stomached for 2 minutes. From this dilution, other serial dilutions were prepared. Aerobic colony counts were determined by the spread plate method, using nutrient agar (Lab M, Lancashire, UK). Xylose lysine deoxycholate agar (XLD, Lab M, Lancashire, UK) was used for enumerating and isolating *Salmonella* spp. Plates were incubated at 37°C for 24 h and the colony forming units (CFU/g) were counted (Al-Harbi and Uddin, 2005; Santo *et al*, 2008). Colonies with different morphologies from nutrient and XLD media were streaked on trypticase soy agar (TSA, Lab M, Lancashire, UK) slants and subjected to biochemical tests, triple sugar iron and identification with API 20E diagnostic strips (Biomérieu, France) as described in published reports (Food and Drug Administration, FDA, 1998; Angela Di Pinto *et al*., 2008).

For counting and isolation of *Vibrio* spp., fish flesh samples (25 g each) were added to a sterile bag with 225 ml of alkaline peptone water (APW, Lab M, Lancashire, UK) with pH 8.6, containing 1% NaCl, stomached for 2 min and incubated overnight at 37°C. Aerobic colony counts were determined by the spread plate method onto thiosulphate-citrate-bile salts-sucrose (TCBS, Lab M, Lancashire, UK) agar plates containing 1% NaCl. Green, blue-green and yellow colonies on TCBS agar plates were presumptively selected as *Vibrio* colonies and transferred to

TSA slants (Lab M, Lancashire, UK) with 2% NaCl (Di Pinto et al., 2008). After incubation, isolates with different morphologies were subjected to the oxidase test using oxidase sticks (Oxoid, Hampshire, UK) and biochemical identification with API 20E as described by the FDA (1998).

Bacterial Identification by sequencing of 16S rDNA

This technique was applied for confirmation of API results and for the identification of unknown bacteria samples. It was performed as described by Janda and Abbott (2007).

Amplification of 16S rDNA

DNA extraction was done using a bacterial DNA preparation kit (Jena Bioscience, Thuringia, Germany). Partial 16S rDNA was amplified using universal oligonucleotides primers (Forward: 5′-GAGTTTGATCCTGGCTTAG-3′and Reverse: 5′-GGTTACCTTGTTACGACTT-3′). Briefly, 2 µl DNA templates (20 ng/ µl) was added to 12.5 µl Master Mix (Qiagen, Hilden, Germany) and 10.5 µl deionised water (H_2O) for a total volume of 25 µl. The mixture was then amplified in a DNA thermal cycler (Techne Progene, Marshall Scientific, Hampton, NH) using the following program: one denaturation step at 94°C for 5 min; 37 cycles (30s at 94ºC, 30s at 51ºC and 30s at 72ºC); and a final extension for 5 min at 72ºC.

Gel preparation and analysis of the PCR products were performed by gel electrophoresis using 1.5% agarose gel with 1X TAE buffer.

DNA Sequencing

Purification of the PCR products was performed using a QIAquick Kit (Qiagen, Hilden, Germany). The second PCR was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (ThermoFisher, MA, USA). Each reaction (20 µl) contained Terminator ready reaction mix (8 µl), primer (3.2 pmol), DNA template quantised according to the PCR product size and deionised water. The thermal profile for cycle sequencing PCR was 1 min at 96ºC; 25 cycles as follows: 10s at 96ºC, 5s at 50ºC and 4 min at 60ºC. After an additional purification step using CENTRI-SEP Columns (PrincetonSeparations, Freehold, NJ), DNA sequencing was carried out by a 3500 Genetic Analyzer (Applied Biosystems, Massachusetts, USA). The obtained consensus sequences were subjected to a BLAST search through the Mega program (7.0.20) as described by Azwai1 *et al.*(2016).

Heavy Metals Analysis

Muscle tissues (0.5 g) of the samples, triplicate samples from each treatment group, were placed into a screw digestion cup before adding nitric acid (HNO_3, 5 ml) and perchloric acid ($HClO_4$, 2 ml) (UNEP/FAO/IAEA/IOC, 1984).Mixtures were heated at 80°C until completely dissolved. After cooling to room temperature, the content was decanted into falcon tubes and the total volume of the sample was made to 25 ml using ultra-pure water. The concentrations of cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni) and lead (Pb) in the digested samples were determined using an Atomic Absorption Spectrophotometer, Shimadzu 6800 (UNEP/FAO/IAEA/IOC, 1984).

Mercury (Hg) analysis was conducted using cold vapor atomic absorption. Dried samples (0.5 g) were weighed, mixed with HNO_3 (5 ml) and $HClO_4$ (2 ml) then heated at 40°C until the samples were dissolved. After cooling at room temperature, ultra-pure water was added to the samples to make the volume 25 ml. Cold vapor atomic absorption spectroscopy was used to determine Hg concentration in the digested sample and the analysis was carried out in triplicate samples (UNEP/IAEA 1984).

Standard reference material (DORM-4: fish protein, the National Research Council of Canada) was used to validate the analysis. Mean recoveries of Hg, Cd, Cr, Pb, Cu and Ni were 96.8, 95.4, 96.1, 95.2, 106.2 and 95.3%, respectively.

Statistical Analysis

Statistical analysis was performed using Minitab® Software (Version 16.0, Minitab Inc., Pennsylvania, USA). All measurements were carried out using seven independent replicate samples. One-way analysis of variance (ANOVA) was used to determine the effects of processing on the measured parameters. Significant differences among the means were determined using Tukey multiple comparison tests in the general linear model (GLM) protocol at a confidence level of 95% ($P < 0.05$).

RESULTS AND DISCUSSION

Microbiological analysis

Aerobic colony counts

Aerobic colony counts (ACC) in fresh chilled fish samples (5.1± 0.2 $\log_{10}$ CFU/g) were within the acceptable limits compared to the Egyptian Organisation for Standardisation, EOS (<10^6CFU/g, EOS, 2005), as shown in Table1. Marinating and salting processes decreased the total count to 1.1± 0.3 and 3.8± 0.2 $\log_{10}$ CFU/g, respectively, demonstrating a reduction of 4 logs detected for fish marinated with 2.5% acetic acid and 3% NaCl while salting reduced the bacterial count by 1.3 logs. The higher reduction in ACC of the marinated fish might be due to combined actions of steaming and the presence of acid and salt in the marinating solutions compared to the effect of salt only at high pH (6.5) in the salted fillet. Similar results of ACC reduction of 1.58 and 1.76 logs were detected in Pacific saury fish marinated with pre-chilled 2% acetic acid or 3% acetic acid and 12% salt, respectively at the end of three months storage (Sallam, 2008). Ozogul *et al*. (2009) reported a reduction in ACC after 90 days (0.3 log reduction, from 3.5 to 3.2 log CFU/g) in Tench after application of warm marinating process with a 4% acetic acid solution. Marinating seabass using 0.1% citric acid and 8% NaCl at 4°C was reported to reduce the number of enterobacteria and hydrogen sulfide (H_2S)-producing bacteria over 3 days of storage, while with prolonged storage, ACC and lactic acid bacteria were slightly increased (Giuffrida *et al*., 2007). On the other hand, Shiriskar *et al*. (2009) reported an increase in the total bacterial count of pickled anchovy from an initial value of 4.85×10^1 to 3.0×10^5 CFU/g after 15 weeks of storage. The fast marinating time trial in this study (only 3 days) was not enough for deep penetration of the acid into the fish flesh, as the pH values of marinating solution and marinated flesh were 4.0 and 5.5, respectively;this suggests potential for further reduction upon extended storage. The low pH of the solution might explain the absence of bacterial growth. Sallam (2008) reported that ACC was significantly ($P < 0.05$) decreased in marinated Pacific saury fish fillet after 40 days of storage.

Table 1: Aerobic colony count $\log_{10}$ (CFU/g) and pH results of fresh fish and processed fish products

	Fresh	Marinated		Salted	Canned		
		Marinated fish	Marinated solution		Tomato sauce	Brine	Oil
Total plate count	5.1 ± 0.2	1.1 ± 0.3	< 1 estimated	3.8 ± 0.2	< 1 estimated	< 1 estimated	< 1 estimated
Salmonella spp.	4.1 ± 0.1	1.0 ± 0.1	< 1 estimated	2.3 ± 0.4	< 1 estimated	< 1 estimated	< 1 estimated
Vibrio spp.	4.4 ± 0.3	< 1 estimated	< 1 estimated	3.7 ± 0.2	< 1 estimated	< 1 estimated	< 1 estimated
pH	6.5 ± 0.1	5.5 ± 0.1	4.0 ± 0.1	6.5 ± 0.1	4.8 ± 0.1	5.1 ± 0.1	4.9 ± 0.1

The values represent the means of seven independent samples (n = 7) ± standard deviation.

In the canning process, fish were exposed to extensive thermal treatment that led to no detection of bacterial growth. Excluding anomalies in the thermal treatment process of canned fish products and any contamination after treatment, the canned fish product should be safe for consumption. Casalinuovo *et al*. (2015) reported that contaminations in canned products were most likely due to inappropriate production quality standards and/or the quality of the raw material used. Our results show that even with high total count raw material, canning can help to improve the microbial count of the fish.

Salmonella

Salmonella is often the etiological agent of gastroenteritis associated with the consumption of contaminated fish (Rahimi *et al*., 2013). It is well established that aquatic birds, raw sewage and pre-harvest contamination from animal and human reservoirs can spread salmonellae and other human pathogens into seafood (Huss *et al*., 2000). *Salmonella enterica* Ras3 was isolated from fresh fish, identified, sequenced and represented in a phylogenic tree in Figure1. The nucleotide sequence of *S. enterica* Ras3 was submitted to Gen-Bank and has been provided by accession number KY120327. *S.enterica* has been reported in different types of fish in different countries: in freshly caught tilapia and catfish in Malaysia; whiting fish in France; European plaice, Atlantic salmon and rainbow trout in Great Britain; European pilchard Portugal; and Nile tilapia in Egypt (Youssef *et al*., 1992; Pullela *et al*., 1998; Davies *et al*., 2001; Budiati *et al*., 2013; Novoslavskij *et al*., 2016). According to EOS standards, *Salmonella* should not be found in chilled fresh fish and its presence in samples in the present study suggests better control and monitoring is required (EOS, 2005).

Table 2: Identification of selected colonies based on API 20E biochemical tests

Fresh fish	Marinated fish	Salted fish
Enterobacter cloacae	-	-
Salmonella enterica	*Citrobacter koseri*	-
Pasteurella pneumotropica	-	*Shewanella putrefaciens*

Processing of fish is an important step to improve food safety. *Salmonella* was not found in any of the processed fish products (marinated, salted or canned fish products). Low ACC (1 log CFU/g) was found on XLD agar for marinated fish. All presumptive colonies were selected and some were identified as *Citrobacter koseri* (Table2). Ozogul *et al*. (2008) reported similar results, where *Salmonella* and other pathogenic bacteria were not detected in marinated fish products while aerobic colony counts remained at 3 logs CFU/g after 3 months of storage. In the salted fish product, the aerobic colony counts were 2.3 logs CFU/g on XLD agar, but no *Salmonella* was found. In contrast, Arkoudelos *et al*. (2003) reported that *S.enteritidis* survived for 90 days in salted sardines during ripening. Similarly, *S. enteritidis* survived in salted horse mackerel fillets after 60 days of salting (salt/fish=80/100) and 65 days (salt/fish=30/100) during storage at 4°C (Mol *et al*., 2010). The differences in the processing (samples were air dried in Mol *et al*. (2010) compared with vacuuming the samples and retaining any exudates in the present study), the high inoculation count in Arkoudelos *et al*. (2003) and Mol *et al*. (2010) or the analysis (XLD was used in all studies, but confirmation using 16S rDNA was done in the present study only) may explain the differences observed.

3.1.3 Vibrio

Vibrio spp. are major food-borne pathogens. High levels of *Vibrio* contamination in raw seafood may be expected because of natural contamination from the aquatic environment. *V. parahaemolyticus* is frequently isolated and reported from seafood in various countries (Wong *et al*., 1999; Basti *et al*., 2006; Nakaguchi, 2013; Novoslavskij *et al*., 2016).

In this study, high ACC were observed on TCBS medium with fresh fish (4.4 logs CFU/g) and salted fish (3.7 logs CFU/g), as shown in Table1. The ACC in salted fish might be due to the growth of halophilic bacteria after 2 weeks storage (Ali, 2012). All suspected colonies were isolated and identified as *Pasteurella pneumotropica* in fresh fish and *Shewanella putrefaciens* in salted fish, with a complete absence of *Vibrio* species (Table2). Ekhtiarzadeh *et al*. (2012) reported that the growth of *V. parahaemolyticus* was inhibited in salted fish fillets (brine salted at 4% NaCl) at 8°C, with a reduction from 3 log CFU/g to <2 log CFU/g at the first day of study. The results of this study are in contrast with reports of long-term survival of *Vibrio* spp. at salt concentrations similar to those of the seawater environment (3.3%) (Armada *et al*., 2013), salted herring (6.2%) and salted roe samples (1.3%) (Chițu *et al*., 1977). No viable bacterial colonies were detected in marinated and canned products. Sallam *et al*. (2008) reported similar results where *Vibrio* was not detected in marinated Pacific saury treated with marinating solutions containing either 12% NaCl and 2% acetic acid or 12% NaCl and 3% acetic acid and stored at 4°C for 90 days. *V. parahaemolyticus* was also reported to be reduced to below detection limits (~5-log reduction) with the use of lime juice for marinating (Mathurand *et al*., 2013). Finally, there are no records of association of *Vibrio* spp.with canned food.

Heavy metals

The heavy metals concentrations of fresh, dry-salted, hot-marinated and canned fish in oil, brine or tomato sauce are shown in Table 3. The average concentrations of Hg, Cd, Cr, Pb, Cu and Ni in fresh fish were below the limit of detection (LOD): 0.16, 0.38, 0.92, 2.25, and 0.55 µg/g, respectively. After processing, the averages of these heavy metals ranged from LOD to 0.01, 0.11 to 0.15, 0.30 to 0.39, 0.83 to 0.91, 2.20 to 2.27 and 0.41 to 0.54 µg/g for Hg, Cd, Cr, Pb, Cu and Ni, respectively (Table 3). In general, hot marinating, dry salting and canning tended to decrease the means of all determined heavy metals, probably due to loss of minerals to the liquids used in processing or the salting out and subsequent proteolysis that occured during salting for two weeks. These reductions were significant only in Cr and Ni (Table 3).

Table 3: Effects of processing methods on heavy metal concentrations (µg/g wt) in processed Indian mackerel

Sample		Hg	Cd	Cr	Pb	Cu	Ni
Raw fresh fish		LOD	0.16 ± 0.01	0.38 ± 0.01	0.92 ± 0.01	2.25 ± 0.01	0.55 ± 0.01
Dry salted fish		LOD	0.14 ± 0.02	0.30 ± 0.04	0.86 ± 0.01	2.20 ± 0.06	0.45 ± 0.01
Hot marinated fish		LOD	0.11 ± 0.01	0.30 ± 0.03	0.85 ± 0.01	2.21 ± 0.02	0.47 ± 0.02
Canned in	**oil**	0.01 ± 0.01	0.12 ± 0.01	0.39 ± 0.01	0.89 ± 0.01	2.24 ± 0.02	0.54 ± 0.01
	brine	LOD	0.14 ± 0.01	0.31 ± 0.01	0.91 ± 0.01	2.27 ± 0.02	0.44 ± 0.01
	tomato sauce	LOD	0.15 ± 0.01	0.33 ± 0.01	0.83 ± 0.01	2.21 ± 0.02	0.41 ± 0.01

LOD = below level of detection.
The values represent the means of seven independent samples (n = 7) ± standard error of the means.

According to the legal standard recommended by the European Commission (EC, 2001), EOS (2004) and FAO (1983), raw fresh fish and their product samples exceeded the maximum residue levels (MRLs) for their Pb and Cd contents (0.5 and

0.1 ppm, respectively). This is of concern, considering that Pb and Cd are toxic and that their accumulation may lead to human health hazards, especially for children.

Lead and Cd are among of the most toxic, widely-distributed metals that accumulate in the food chain and are easily absorbed during digestion (Krejpcio and Trojanowska, 2000; Dermirezen and Uruç, 2006). Berny *et al*. (1994) and the Commission of the European Communities (2001) stated that these metals can cause serious health problems. Pb may cause problems in the nervous and urinary systems and inhibit heme synthesis, while Cd may lead to the development of prostate and breast cancer and skeletal abnormalities (Nordberg *et al*., 2002; Saha and Zaman, 2012).

In Egypt between 1996 and 2010 fish consumption increased from 8.5 kg to 16.7 kg/person/year (FAO, 2010). According to the FAO (2010), the average quantity of fish consumed per person (assuming a 70 kg person) per day in Egypt was 45.75 g/person/day. The estimated daily intakes (EDI) or weekly intakes (EWI) and EWI/provisional tolerable weekly intake (PTWI) ratio for Pb and Cd in fresh and processed fish products are shown in Table 4. The EDI or EWI for Pb and Cd in fish samples in this study were estimated according to the following equation:

$$EDI = C_{metal} \times W/bw$$

Where C_{metal} is the average concentration of Pb and Cd in fish; W represents the daily average consumption of marine fish; and bw is body weight, set to 70 kg. PTWI standard levels were provided by the European Food Safety Authority (EFSA, 2009 & 2010). The results of EWI and EDI values were below the recommended EFSA values for Pb and Cd (25 and 2.5 µg/kg bw, respectively) (Table4).

Table 4: The estimated daily intake (EDI, µg/70kg bw/day) and estimated weekly intake (EWI,µg/70kg bw/week) and estimated weekly intake/provisional tolerable weekly intake (EWI/PTWI) ratio of Pb and Cd of fresh fish and canned fish in oil, brine and tomato sauce

Sample		**Pb**				**Cd**			
		Conc.	**EDI**	**EWI**	**EWI/ PTWI**	**Conc.**	**EDI**	**EWI**	**EWI/ PTWI**
Raw fresh fish		0.92	0.32	2.21	0.09	0.16	0.05	0.38	0.15
Raw fresh fish		0.86	0.29	2.06	0.08	0.14	0.05	0.34	0.13
Hot marinated fish		0.85	0.29	2.04	0.08	0.11	0.04	0.26	0.11
Canned in	**oil**	0.89	0.31	2.14	0.09	0.12	0.04	0.29	0.12
	brine	0.91	0.31	2.18	0.09	0.14	0.05	0.34	0.13
	tomato	0.83	0.28	1.99	0.08	0.15	0.05	0.36	0.14

Calculated from the EFSA values, 25 and 2.5 µg/kg bw, respectively.

The EWI/PTWI ratios for Pb and Cd in fish samples were below 1, which means that the results remained within the established safety regulations for humans. The results of this study agree with those of Elisavet *et al*. (2014), who found that in Egypt the mean values for Pb and Cd content in demersal fish (1.74 and not recorded (NR) µg/g, respectively), pelagic fish (1.7 and 0.19 µg/g, respectively) and marine fish (1.46 and 0.75 µg/g, respectively) were high. The EWI values were also high (2.09 and NR; 1.70 and 0.19; and 0.87 and 0.45µg/week/70 kg bw) in demersal, pelagic and marine fish, respectively, while the EWI/PTWI data ratios were low (0.08 and NR; 0.07 and 0.08; and 0.03 and 0.18, respectively).

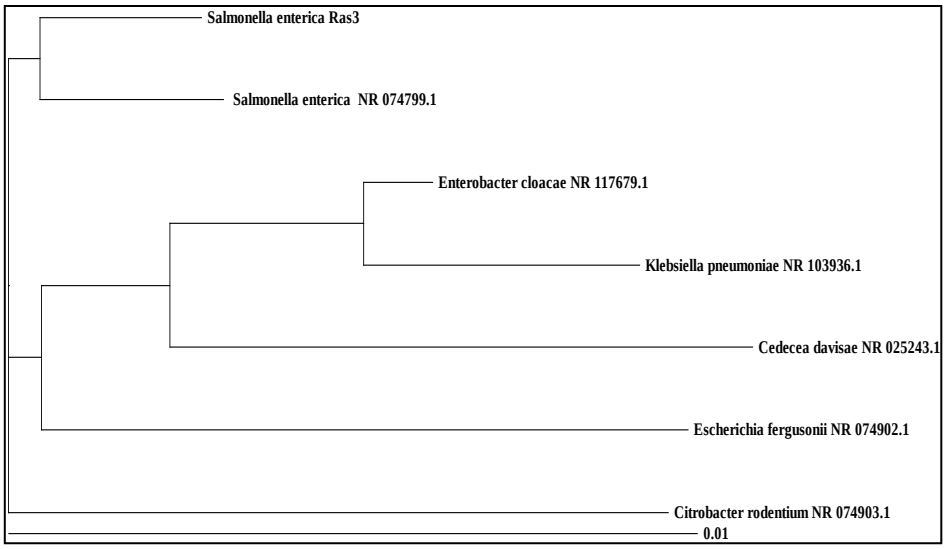

Fig. 1: Phylogenic tree of sequenced *Salmonella enterica* RAS3.

CONCLUSION

This study showed that the processing of Indian mackerel fish (cascomry) by salting (using 15%dry salt) and the hot marinating process (steaming and marinating in a solution containing 2.5% acetic acid and 3% NaCl) improved the microbiological quality of the product and inhibited the growth of food-borne pathogens. Canning with hot oil, brine (3%) or sauce (8% TSS) eliminated all viable bacterial growth. Moreover, processing did not cause an increase in heavy metal content compared to fresh fish. Fresh fish and their products exceeded the maximum standard residue levels for Pb and Cd contents; however, results of estimated daily and weekly values were below the recommended safety margins for human consumption. Therefore, these fast processing techniques can be used as safe methods for the preservation of cascomry fish in Egypt.

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