Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

The change in heat inactivation of *Escherichia coli* O157:H7 after entering into the viable but non-culturable state in salted fish, *Hypophthalmichthys molitrix*

Mohammad Khezri[1], Masoud Rezaei[1]*, Ashraf Mohabbati Mobarez[2], Mehdi Zolfaghari[3]**

1. Department of Seafood Science, Faculty of Marine Sciences, Tarbiat Modares University, Noor, Iran
2. Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
3 Fisheries Department, Agriculture and Natural Resource University of Gorgan, Gorgan, Iran

** Corresponding author's E-mail: rezai_ma@modares.ac.ir*
*** Corresponding author's E-mail: mmmobarez@modares.ac.ir*

ABSTRACT

Many species of non-sporulating bacteria including *Escherichia coli* can enter into a viable but non-culturable (VBNC) state under stress conditions. In this study, the change in thermal resistance of *E. coli* O157:H7 after entering into the VBNC state in salted silver carp, *Hypophthalmichthys molitrix*, was investigated. *E. coli* O157:H7 was inoculated on the fish control group (T_F) and on those fish with 30% NaCl ($T_{F + 30\% NaCl}$) at room temperature. Culturability of bacteria was determined using routine culture and colony counting on Sorbitol MacConkey agar. When bacteria were non-cultivable, the RT-PCR of 16S rRNA gene (including direct extraction and purification of RNA, DNase I treatment for removing DNA contamination, cDNA synthesis and electrophoresis of PCR products of cDNA) was used to detect VBNC *E. coli* O157:H7. Also, cultivable and VBNC *E. coli* O157:H7 were individually heat-treated at 55, 62 and 70 °C for 5 min. The samples were cooled and after 24 h, the thermal resistance of bacteria was determined through viability detection using RT-PCR of 16S rRNA gene. The culturability of bacteria was kept in fish treatment but they were non-cultivable in fish under 30% NaCl after 5 days. The positive expression of 16S rRNA in all studied treatments indicated the entering of *E. coli* O157:H7 into the VBNC state in fish treatment under 30% NaCl. Moreover, the RT-PCR of 16S rRNA gene showed that only VBNC forms of *E. coli* O157:H7 showed viability at 62 °C for 5 min which indicated the increased resistant of VBNC bacteria to the thermal inactivation. The ability of *E. coli* O157:H7 to enter into VBNC state in salted fish and also the increase of its thermal resistance suggest that VBNC *E. coli* O157: H7 can be considered as critical threat to public health and food safety.

Keywords: *Escherichia coli* O157:H7, VBNC state, Thermal resistance.

Article type: Research Article

INTRODUCTION

Salting and smoking are popular and inexpensive methods for producing preferable taste and color as well as for preserving meat and fish in many parts of the world, however, good hygiene is rarely practiced during producing traditional salted fish especially in rural areas (Yam *et al.* 2015). Pathogenic bacteria can contaminate seafood through the fecal contamination of water and also unhygienic conditions during the handling processes (Costa 2013; Khezri *et al*. 2016). Silver carp (*Hypophthalmichthys molitrix*) is among the most important economical species which is widely sold and used as fresh, however, its traditional salted and smoked products have

Received: April 19, 2021 Accepted: Aug. 10, 2021

Publisher: University of Guilan, Iran

popularized in Iran (Akhondzadeh Basti *et al.* 2006; Razavilar *et al.* 2013; Shi *et al.* 2017). Silver carp is polycultured with other carp species in aquaculture ponds in which animal manure is used as fertilizers, so serious concerns are raised about the presence of pathogenic bacteria such as *Escherichia coli* in the final salted products (Khezri *et al*. 2020). Greenlees *et al*. (1998) identified *E. coli* as inhabitants in pond water and isolated these bacteria in fish from those ponds (Greenlees *et al.* 1998). In this regard, several studies reported the presence of *E. coli* in fresh fish, however, no *E. coli* was detected after salting process (Akhondzadeh Basti *et al.* 2006; Tavakoli *et al.* 2012; Razavilar *et al.* 2013). This raise again the concern about the entering of *E. coli* into the viable but non-culturable (VBNC) state under high salinity stress (Oliver 2010; Khezri *et al.* 2020). VBNC is a physiological state in which many non-sporulating bacteria under various stress conditions lose their culturability on routine culture media, but remain viable with some metabolic activity for long periods of time (Castro-Rosas *et al.* 2017; Zolfaghari *et al*. 2020). VBNC bacteria maintain their pathogenic potential and continue to express infectious gene in the VBNC state or after resuscitation in favorable condition (Makino *et al.* 2000; Li *et al.* 2014; Castro-Rosas *et al.* 2017; Zhao *et al.* 2017). In this regard, several foodborne disease outbreaks have been associated with contamination of fish or fish products with VBNC state of *E. coli* O157:H7. For instance, an outbreak occurred due to the consumption of salted salmon roe contamination with *E. coli* O157 in the VBNC state (Makino *et al.* 2000). In this regard Salmonella and Shigella contaminations were also reported in fish and fishery products including salted fish (Yam *et al.* 2015) and *E. coli* has been reported to be responsible for the production of histamine in the dried fishes (Logesh *et al.* 2012). So, critical cautions have to be taken about the safety of salted and smoked fish product that mostly consumed undercooked. VBNC bacteria are unable to be detected by routine culture and colony counting methods. Since one of the differences between VBNC cells and dead cells is maintaining genetic information and gene expression in these cells (Oliver 2010), and also regarding the short stability of RNA compared with DNA, the Reverse Transcription-PCR (RT-PCR) of bacterial housekeeping genes can be used as accurate known indicator for determining the viability of bacteria (Li *et al.* 2014; Zolfaghari *et al*. 2020; Khezri *et al*. 2020).

Despite the available information supporting the VBNC state of bacteria as a survival and resistant mechanism against the unfavorable condition, the change in their resistance against inactivation treatments continue to be debatable. Mukherjee *et al.* (2008) reported that citric or acetic acid treatments caused the decrease of heat resistance of *E. coli* O157:H7 in beef products (Mukherjee *et al.* 2008). On the other side, it has been reported that if heating process is too slow, heat resistance of *E. coli* O157:H7 at 55, and 60 ˚C in culture broth and ground beef was increased by 2- and 1.5-fold, respectively (Murano & Pierson 1992; Juneja *et al.* 1998). Few investigations are conducted on the thermal inactivation of pathogenic bacterial including *E. coli* O157:H7, however, as we know there is no study investigating the heat inactivation of VBNC *E. coli* O157:H7 in salted fish. The aim of this investigation was to determine if entering of *E. coli* O157:H7 into the VBNC stat increase heat resistance in VBNC cells.

MATERIALS AND METHODS

Preparation of bacterial culture

The present study was carried out in seafood processing laboratory and medical bacteriology laboratory in Tarbiat Modarres University, Iran during 2016-18. *Escherichia coli* O157:H7 which was isolated and confirmed (*Eschericia coli* O157:H7, Isolate Number: 295) by the Microbiology Department, Veterinary Faculty, Tehran University, Iran was used in this study. *E. coli* O157:H7 was grown in BHI broth for 24 h at 37 °C at 120 rpm. This overnight bacterial culture was further grown in BHI broth. To obtain the bacterial growth curve, bacterial growth was monitored using a spectrophotometer at OD600 nm. Bacterial cultures were also grown on BHI agar and after incubating for 24 h at 37 °C the bacterial counting was performed on plates containing 30-300 colonies and the results were reported as CFU mL^{-1}. The bacteria in the early phase of bacterial growth were centrifuged (5000g for 15 min) and the harvested cells were washed twice with physiological serum (0.85% NaCl). The washed inoculum was resuspended in physiological serum and used for inoculation into the studied treatments (Sue *et al.* 2003; Khezri *et al*. 2019).

Inducing VBNC state of *E. coli* O157:H7

E. coli O157:H7 at early log phase were inoculated on silver carp fillet (control group = T_F) and silver carp fillet to which 30% NaCl was added (treatment F+30% NaCl = $T_{F+30\%\ NaCl}$) at room temperature. Silver carp were bought from the local market and were transferred to the laboratory on ice. Then the fish were filleted, divided

into pieces of about 10 g and were placed in sterile petri plates. Then, 1 mLof the prepared bacterial suspension to reach the final concentration of 5×10^7 CFU g^{-1} was inoculated to each piece of meat. Contaminated meat in sterile petri plates was placed for 1 h at room temperature under the sterile hood in order to provide enough time for bacteria to stabilize on the fillet. Control group and $T_{F+30\% NaCl}$ were prepared and the prepared samples were kept at room temperature, followed by sampling at days 0, 1, 2, 3, 4, 5, 7, 9 and 11. In order to investigate the bacterial cultivability in studied treatment, serial dilution was prepared and bacterial samples were cultured on Sorbitol MacConkey agar. Bacterial counting was determined and reported as CFU mL^{-1}. In order to determine the status of cultivated bacteria at the end of the experimental period, when the number of cultivable bacteria was less than 0.1 CFU mL^{-1}, the bacteria were considered in VBNC state (Liu *et al.* 2008). In order to investigate the presence of viable bacteria in different treatments, 16S rRNA RT-PCR was used, including extraction of RNA, DNase I treatment for removing DNA contamination, followed by cDNA synthesis and electrophoresis of PCR products of prepared cDNAs (Tan *et al.* 2012).

RT-PCR of 16S rRNA

The homogenized samples were centrifuged at 7000 rpm for 5 min. The pellets containing the bacteria were used for cells lyses and RNA extraction. So, the RiboEx Total RNA kit (GeneAll, South Korea) was used according to the protocols of the manufacturer Company. DNase I Kit (Sinaclon, Iran) was used to remove the DNA pollution. The resulting RNA was used for cDNA synthesis using Viva 2-step RT-PCR kit (Vivantis, Malaysia). Then PCR was performed. In order to perform PCR, the Prime Taq Premix (2X) (Qiagen Tebe Sadra, Iran) was used. Briefly, 10 µL Prime Taq Premix, 3 µL DNA template and 1 µL mixed forward and reverse primers (each 5 picomolar) were mixed and the final volume was reached to 20 µL using sterile deionized water. The mixture was partially centrifuged for 10 seconds to remove the droplets on the wall of micro-tubes. Then the micro-tubes were placed in PCR machine. PCR cycling conditions included: 1) primary denaturation: 94 °C for 5 min; 2) replication of DNA for 35 cycles including A) secondary denaturation for 30 seconds at 94 °C, B) annealing of primers for 30 seconds at 60°C and C) extension for 30 seconds at 72 °C; 3) final elongation at 72°C for 5 min. The electrophoresis of PCR products was performed using agarose gel (2%) and the gel was photographed using a Gel Doc. The sequences of primers of 16S rRNA gene used in this study were as forward primer: CATTACGTTACCCGCAAA and reverse primer: CCTTTACGCCCAGTAATTCC with a final PCR product size of 98 bp.

Evaluation of the changes in thermal inactivation

Both cultivable and VBNC forms of *E. coli* O157:H7 obtained respectively in the T_F and $T_{F+30\% NaCl}$ treatments at the end of study period were heat-treated at 55, 62 and 70 °C for 5 min by immersing the 50 mL-falcon tubes containing above treatments in a circulating water bath. Immediately after heat treatment, the samples were cooled in ice bath for 10 min to stop further effects of heat, followed by keeping for 24 h at room temperature. Then samples were used for RT-PCR of 16S rRNA gene to determine the viability of bacteria in the studied treatments. Moreover, the freshly-grown *E. coli* O157:H7 in BHI broth was used at the same temperature conditions to compare the response of the bacteria to the heat inactivation in different treatments. In addition, in the case of heat inactivation of freshly-grown *E. coli* O157:H7, it can be used as good control for the efficacy of RT-PCR to differentiate viable bacteria from dead ones (Tan *et al.* 2012; Juneja *et al.* 2003; Juneja *et al.* 1998).

Statistical processing and data analysis

The experiments were conducted in a completely randomized design. All tests were carried out in triplicate. Mean and standard deviation of data were determined and used to draw charts using excel software. The results of 16S rRNA gene expression using RT-PCR were reported qualitatively and the picture of PCR electrophoresis was presented on 2% agarose gel.

RESULTS

In this study, *E. coli* O157:H7 was inoculated on silver carp fillet (T_F) and silver carp to which 30% NaCl was added ($T_{F+30\% NaCl}$) at room temperature and its culturability was measured using routine culture and colony counting method. The results showed that the culturability of inoculated bacteria in T_F was maintained throughout the study period (11 days), but their culturability was lost on day 5 under salinity of 30% ($T_{F+30\% NaCl}$; Fig. 1).

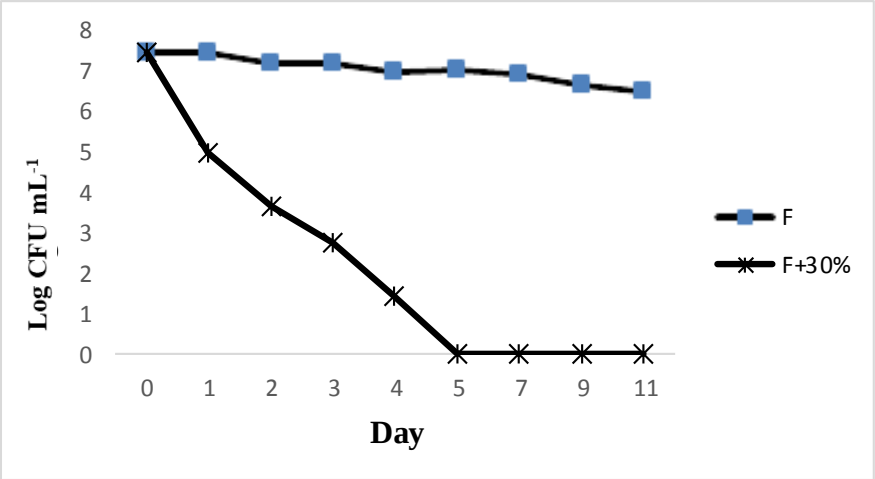

Fig . 1. The changes in culturability of *E. coli* O157:H7 in fish fillet (T_F) and fish fillet under 30% NaCl ($T_{F+30\%NaCl}$) at room temperature.

In the case of no culturability, RT-PCR of 16S rRNA was used to detect the presence of viable *E. coli* O157:H7. As shown in Fig. 2, the extracted RNA treated with DNase I was negative for the expression of 16S rRNA, indicating that all DNA contaminations and subsequent false positive results were eliminated.

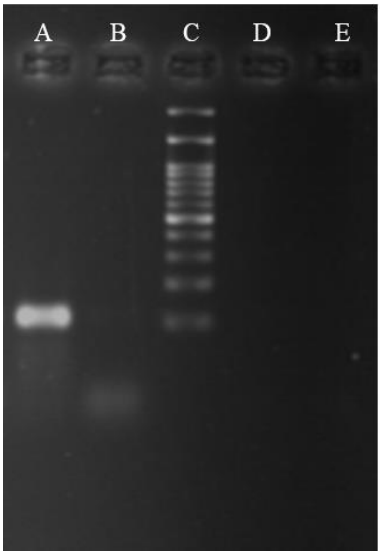

Fig. 2. DNase I treatment for removing DNA contamination in extracted RNA for RT-PCR of 16S rRNA in culturable and non-culturable *E. coli* O157:H7; A: positive control, B: negative control, C: DNA ladder, D: DNAse I treated of extracted RNA from culturable bacteria, E: DNAse I treated of extracted RNA from non-culturable bacteria.

As shown in Fig. 3 and Table 1, the expression of 16S rRNA gene was persisted in both studied treatments throughout the study period (even in $T_{F+30\%NaCl}$ up to day 11), which indicated the viability and entering the bacteria into the VBNC state in $T_{F+30\%NaCl}$ (Fig. 3; Table 1).

In this study, freshly-grown in BHI broth, cultivable and VBNC *E. coli* O157:H7 showed positive expression of *16S rRNA* gene at 55 ºC for 5 min (Fig. 4), indicating the viability of bacteria under this thermal condition. However, at 62 ºC for 5 min only VBNC *E. coli* O157:H7 exhibited positive expression of *16S rRNA* gene (Fig. 5), indicating the increased thermal resistance in VBNC form of this pathogen compared with the cultivable forms. At 70 ºC for 5 min, all studied forms of *E. coli* O157:H7 were negative for the expression of *16S rRNA* gene (Fig. 6) indicated that no bacteria remained viable under this thermal inactivation treatment.

Table 1. The expression of *16S rRNA* of *E. coli* O157:H7 using RT-PCR fish fillet (T_F) and fish fillet under 30% NaCl ($T_{F+30\%NaCl}$).

Treatment / Time (day)	F	F + 30% NaCl
0	+	+
1	+	+
2	+	+
3	+	+
4	+	+
5	+	+
7	+	+
9	+	+
11	+	+

+ indicating the positive expression of *16S rRNA* gene; - indicating the negative expression of *16S rRNA* gene

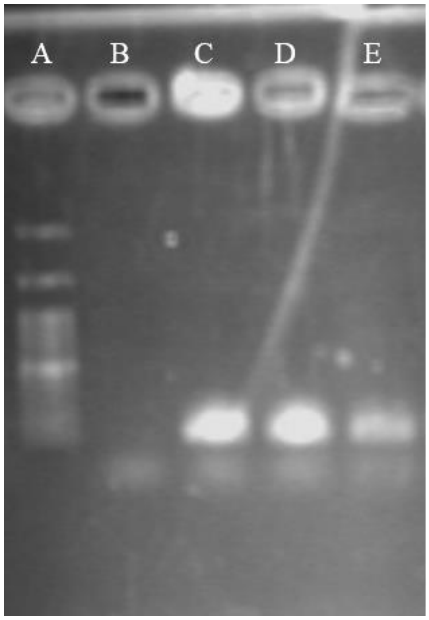

Fig. 3. RT-PCR of 16S rRNA in culturable and non-culturable *E. coli* O157:H7; A: DNA ladder, B: negative control, C: positive control, D: culturable bacteria (T_F on day 11), E: non-culturable bacteria ($T_{F+30\%NaCl}$ on day 11).

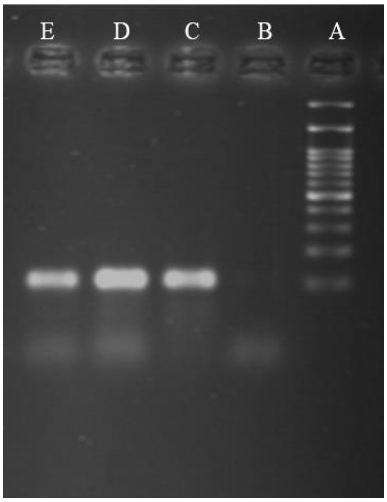

Fig. 4. Thermal resistance of different states of *E. coli* O157:H7 at 55 ºC for 5 min using RT-PCR of *16S rRNA*
A: DNA ladder, B: negative control, C: freshly grown in BHI broth, D: culturable, E: VBNC *E. coli* O157:H7

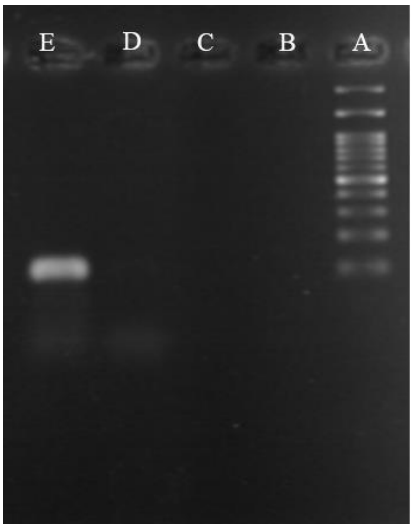

Fig. 5. Thermal resistance of different states of *E. coli* O157:H7 at 62 ºC for 5 min using RT-PCR of *16S rRNA* A: DNA ladder, B: negative control, C: freshly grown in BHI broth, D: culturable, E: VBNC *E. coli* O157:H7.

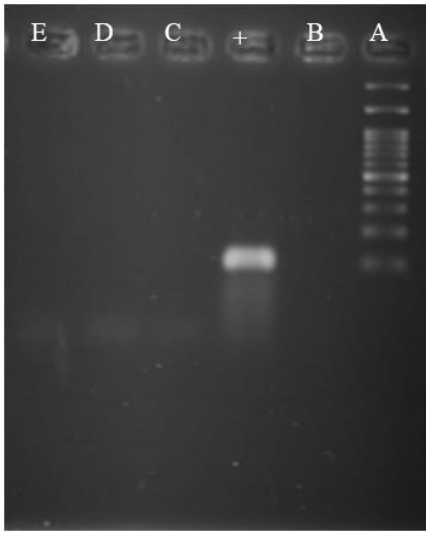

Fig. 6. Thermal resistance of different states of *E. coli* O157:H7 at 70 ºC for 5 min using RT-PCR of *16S rRNA*; A: DNA ladder, B: negative control, +: positive control, C: freshly-grown in BHI broth, D: culturable, E: VBNC *E. coli* O157:H7

DISCUSSION

VBNC state as a survival mechanism of many bacteria has important implications in environmental monitoring, food technology, and infectious disease management. So, studying the VBNC state of pathogenic bacteria and their resistance to inactivation treatments and their association with the water/foodborne outbreaks is of great necessity (Ramamurthy *et al.* 2014). Regarding the documented reports on the pathogenic contamination of raw seafood and also the undercooked consuming of salted fish, proper handling and cooking of seafood is increasingly emphasized. In this study, the change in heat inactivation of *Escherichia coli* O157:H7 after entering into the VBNC state in salted silver carp was investigated. The result of present study showed that *E. coli* O157:H7 under salinity stress of 30% in silver carp lost its culturability after 5 day and its positive expression of *16S rRNA* gene using RT-PCR indicated the viability of these bacteria and their entering into the VBNC state. Our results on entering of *E. coli* O157 into the VBNC state under high salinity stress are in line with the results of other studies. Makino *et al.* (2000) also reported the entering of *E. coli* O157 into the VBNC state under salinity stress in NaCl water (Makino *et al.* 2000). The successful detection of VBNC form of *E. coli* O157:H7 using RT-PCR in this study is in line with the result of previous studies (Coutard *et al.* 2005; Liu *et al.* 2008; Casasola-Rodríguez *et al.* 2018; Khezri *et al*. 2020). This is of great interest as conventional food and water testing methods such as routine culture and colony counting method are unable to detect VBNC state of bacteria. Moreover, one of the differences

between VBNC cells and dead cells is maintaining genetic information and gene expression in these cells (Oliver 2010). However, detection method based on routine PCR are also unable to differentiate viable cells from dead ones. The short stability of RNA in comparison with DNA provide great opportunity for using the Reverse Transcription-PCR (RT-PCR) of bacterial housekeeping genes for determining the viability of bacteria. RT-PCR technology is based on the very short half-life of RNA and the principle that RNA exist only in viable cells (Li *et al.* 2014; Khezri *et al*. 2020). Also, as shown in Fig. 6, heat-killed bacteria (70 ºC for 5 min) were negative for 16S rRNA by RT-PCR after 24 h of heat inactivation. This results again displayed the usefulness of RT-PCR of 16S rRNA for detection of VBNC form of *E. coli* O157:H7. However the accurate stability time of 16s rRNA or other selected genes for using in RT-PCR should be determined to remove the possible false positive results. In this study, it was observed that VBNC state of *E. coli* O157:H7 induced by salinity stress of 30% was more resistant against the thermal inactivation compared to the cultivable forms. In line with our results, Li *et al.* (2016) also reported that salt adopted *E. coli* had higher thermal resistance compared to the non-stressed strains (Li & Gänzle 2016). Also, Osaili *et al.* (2006) reported that addition of salts decreases water activity and increases the thermal resistance of bacteria in chicken-fried and beef patties (Osaili *et al*. 2006). In addition, it was reported that sodium chloride treatment increased thermal resistance of *L. monocytogenes* in ground pork (Line *et al.* 1991) and beef gravy (Juneja & Eblen 1999). Furthermore, it is reported that salts including calcium lactate and potassium lactate can enhance membrane stabilization of bacteria and elevate their thermal resistance (Mukherjee *et al.* 2008).These results are in harmony with our result on the upraising thermal activity of *E. coli* O157:H7 under salinity stress.

The effect of NaCl on increased thermal resistance of bacteria may be due to the binding ability of salt to water in the heating menstruum resulting in poor heat penetration to reach the bacteria (Kotrola & Conner 1997; Juneja 2003; Osaili *et al.* 2006). Moreover, the improvement in the ability of VBNC cells of *E. coli* to resist external mechanical damage in comparison with the cultivable ones may be related to the lower metabolic activity and stronger cell wall strengthened resulting from the increased peptidoglycan cross-linking in VBNC state (Signoretto *et al.* 2002; Zhao *et al.* 2017). In this regard, Signoretto *et al*. (2002) also found three times increase in peptidoglycan DAP–DAP cross-linking in VBNC cells of *E. coli* (Signoretto *et al.* 2002). In line with our results, Ramamurthy *et al.* (2014) found that the VBNC cells of *Vibrio vulnificus* was more resistant to oxidative stress in the presence of H_2O_2 and to 1-chloro-2,4-dinitrobenzene compared to the normal viable cells which may be due to the highly expressed glutathione S-transferase in the VBNC state (Ramamurthy *et al.* 2014).

The difference in bacterial species and its growth conditions such as the growth phase of the cells, composition, pH of the growth medium, heat shock, the difference in environmental or food matrix as well as storage conditions such as pH, storage temperature can affect the thermal resistance of different bacteria (Stringer *et al.* 2000). It is reported that heat resistance was greater when cells were in the stationary (compared to logarithmic) growth phase, grown at 37 or 40°C (compared to 10 or 26°C) and in food with lower fat content. Moreover, treatments with citric and acetic acid resulted in higher reduction of *E. coli* O157:H7 internalized in non-intact beef products and improved its thermal inactivation which indicated the cell injury suffered due to exposure to the organic acids (Mukherjee *et al.* 2008). However, there is no study suggesting *E. coli* o157:H7 as unusually heat-resistant bacteria as compared to other non-spore forming foodborne pathogens (Stringer *et al.* 2000). So the inactivation of all form of studied *E. coli* 0157:H7 at 70 °C for 5 min in our study is acceptable. Heat induces changes in the properties of membrane, cytoplasm, ribosome and DNA, particularly on protein misfolding and aggregations (Li & Gänzle 2016). However, resistant systems of *E. coli* act against these alterations, mainly through gene regulations of heat response, heat shock proteins, refolding of misfolded proteins as well as expression of key proteins of membrane and stabilization of membrane fluidity (Li & Gänzle 2016).

CONCLUSION

Our results demonstrated that food-borne *E. coli* O157:H7 was capable of entering into the VBNC state under high salinity stress during salted fish processing. Moreover, VBNC bacteria were more resistant to the thermal inactivation compared to cultivable forms of these bacteria. However, in nature, bacteria exist in multi-strain combinations, and it is reported that different strains of *E. coli* O157 had different heat resistances at a specific temperature. Also, stress condition and food matrix can have an important role on the behavior of bacteria to response the stresses. So, further studies are needed to determine the possible effects of various stress condition and food matrix properties on thermal inactivation of *E. coli* O157:H7 in the VBNC state. The increased resistance of VBNC *E. coli* O157:H7 to the thermal inactivation highlights the importance of monitoring and development

of detection and inactivation methods of VBNC food-borne pathogens. In the present study, the thermal response of VBNC *E. coli* was investigated under laboratory condition in the 10 g slice of silver carp. However, more cautions must be taken about the whole fish or big fillet.

ACKNOWLEDGEMENT
The authors would like to thank the Iran National Sciences Foundation (INSF) for financial support of the project. Also, we would like to appreciate the authorities of medical bacteriology, seafood processing laboratories and the related specialized departments that helped us in conducting this project.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES
Akhondzadeh Basti, A, Misaghi, A, Salehi, TZ & Kamkar, A 2006, Bacterial pathogens in fresh, smoked and salted Iranian fish. *Food Control*, 17: 183-188.

Casasola-Rodríguez, B, Ruiz-Palacios, GM, Pilar, RC, Losano, L, Ignacio, MR & Orta de Velásquez, MT 2018, Detection of VBNC *Vibrio cholerae* by RT-Real Time PCR based on differential gene expression analysis. *FEMS Microbiology Letters*, 365(15): 1-8.

Castro-Rosas, J, Gómez-Aldapa, CA, Villagómez Ibarra, JR, Santos-López, EM & Rangel-Vargas, E 2017, Heat resistance of viable but non-culturable *Escherichia coli* cells determined by differential scanning calorimetry. *FEMS Microbiology Letters*, 364(19): 1-5.

Costa, RA 2013, *Escherichia coli* in seafood: A brief overview. *Advances in Bioscience and Biotechnology*, 4: 450-454.

Coutard, F, Pommepuy, M, Loaec, S & Hervio- Heath, D 2005, mRNA detection by reverse transcription–PCR for monitoring viability and potential virulence in a pathogenic strain of *Vibrio parahaemolyticus* in viable but nonculturable state. *Journal of Applied Microbiology*, 98: 951-961.

Greenlees, KJ, Machado, J, Bell, T & Sundlof, SF 1998, Food borne microbial pathogens of cultured aquatic species. *Veterinary Clinics of North America: Food Animal Practic*, 14: 101-112.

Juneja, V, Klein, P & Marmer, B 1998, Heat shock and thermotolerance of *Escherichia coli* O157: H7 in a model beef gravy system and ground beef. *Journal of Applied Microbiology*, 84: 677-684.

Juneja, VK 2003, Predictive model for the combined effect of temperature, sodium lactate, and sodium diacetate on the heat resistance of *Listeria monocytogenes* in beef. *Journal of Food Protection*, 66: 804-811.

Juneja, VK & Eblen, BS 1999, Predictive thermal inactivation model for *Listeria monocytogenes* with temperature, pH, NaCl, and sodium pyrophosphate as controlling factors. *Journal of Food Protection*, 62: 986-993.

Khezri, M, Rezaei, M, Rabiey, S & Garmsiri, E 2016, Antioxidant and antibacterial activity of three algae from persian gulf and caspian sea. *Ecopersia*, 4: 1425-1435.

Khezri, M, Rezaei, M, Mohabbati Mobarez, A & Zolfaghari, M 2019, Detection of viable but non-culturable state of *Escherichia coli* O157: H7 using reverse transcription PCR. *Iranian Journal of Medical Microbiology*, 12: 390-398.

Khezri, M, Rezaei, M, Mohabbati Mobarez, A & Zolfaghari, M 2020, Viable but non culturable state and expression of pathogenic genes of *Escherichia coli* O157: H7 in salted silver carp. *Journal of Food Safety*, 40: e12843.

Kotrola, JS & Conner, DE 1997, Heat inactivation of *Escherichia coli* O157: H7 in turkey meat as affected by sodium chloride, sodium lactate, polyphosphate, and fat content. *Journal of Food Protection*, 60: 898-902.

Li, H & Gänzle, M 2016, Some like it hot: heat resistance of *Escherichia coli* in food. *Frontiers in Microbiology*, 7(1763): 1-12.

Li, L, Mendis, N, Trigui, H, Oliver, JD & Faucher, SP 2014, The importance of the viable but non-culturable state in human bacterial pathogens. *Frontiers in Microbiology*, 5(258): 1-20.

Line, JE, Fain, JR, Moran, AB, Martin, LM, Lechowich, RV, Carosella, JM & Brown, WL 1991, Lethality of heat to *Escherichia coli* O157: H7: D-value and z-value determinations in ground beef. *Journal of Food Protection*, 54(10): 762-766.

Liu, Y, Gilchrist, A, Zhang, J & Li, XF 2008, Detection of viable but nonculturable *Escherichia coli* O157: H7

bacteria in drinking water and river water. *Applied and Environmental Microbiology*, 74(5): 1502-1507.

Logesh, A, Pravinkumar, M, Raffi, S & Kalaiselvam, M 2012, An investigation on microbial screening on salt dried marine fishes. *Journal of Food Resource Science*, 1(1): 15-21.

Makino, SI, Kii, T, Asakura, H, Shirahata, T, Ikeda, T, Takeshi, K & Itoh, K 2000, Does enterohemorrhagic *Escherichia coli* O157: H7 enter the viable but nonculturable state in salted salmon roe? *Applied and Environmental Microbiology*, 66: 5536-5539.

Mukherjee, A, Yoon, Y, Belk, KE, Scanga, JA, Smith, GC & Sofos, JN 2008, Thermal inactivation of *Escherichia coli* O157: H7 in beef treated with marination and tenderization ingredients. *Journal of Food Protection*, 71: 1349-1356.

Murano, EA & Pierson, MD 1992, Effect of heat shock and growth atmosphere on the heat resistance of *Escherichia coli* O157: H7. *Journal of Food Protection*, 55: 171-175.

Oliver, JD 2010, Recent findings on the viable but nonculturable state in pathogenic bacteria. *FEMS Microbiology Letters*, 34: 415-425.

Osaili, T, Griffis, C, Martin, E, Beard, B, Keener, A & Marcy, J 2006, Thermal inactivation studies of *Escherichia coli* O157: H7, *Salmonella*, and *Listeria monocytogenes* in ready-to-eat chicken-fried beef patties. *Journal of Food Protection*, 69: 1080-1086.

Ramamurthy, T, Ghosh, A, Pazhani, GP & Shinoda, S 2014, Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria. *Frontiers in Public Health*, 2(103): 1-8.

Razavilar, V, Khani, M & Motallebi, A 2013, Bacteriological study of cultured silver carp (*Hypophthalmichthys molitrix*) in Gilan province, Iran. *Iranian Journal of Fisheries Sciences*, 12: 689-701.

Shi, C, Cui, J, Liu, X, Zhang, Y, Qin, N & Luo, Y 2017, Application of artificial neural network to predict the change of inosine monophosphate for lightly salted silver carp (*Hypophthalmichthys molitrix*) during thermal treatment and storage. *Journal of Food Processing and Preservation*, 41: e13246.

Signoretto, C, del Mar Lleo, M & Canepari, P 2002, Modification of the peptidoglycan of *Escherichia coli* in the viable but nonculturable state. *Current Microbiology*, 44: 125-131.

Stringer, S, George, S & Peck, M 2000, Thermal inactivation of *Escherichia coli* O157: H7. *Journal of Applied Microbiology*, 88(29): 79-89.

Sue, D, Boor, KJ & Wiedmann, M 2003, σB-dependent expression patterns of compatible solute transporter genes opuCA and lmo1421 and the conjugated bile salt hydrolase gene bsh in *Listeria monocytogenes*. *Microbiology*, 149: 3247-3256.

Tan, Q, Xu, H, Chen, T, Li, P, Aguilar, ZP, Xu, D, Ming, X, Xu, F & Wei, H 2012, Differential expression of virulence and stress fitness genes during interaction between *Listeria monocytogenes* and *Bifidobacterium longum*. *Bioscience, Biotechnology, and Biochemistry*, 76: 699-704.

Tavakoli, H, Soltani, M & Bahonar, A 2012, Isolation of some human pathogens from fresh and smoked shad. *Iranian Journal of Fisheries Sciences*, 11: 424-429.

Yam, B, Morteza, K, Sahar, A & Maryam, S 2015, Microbial quality of salted dried fish sold near the Caspian Sea. *Iranian Journal of Basic Medical Sciences*, 2: 61-65.

Zhao, X, Zhong, J, Wei, C, Lin, CW & Ding, T 2017, Current perspectives on viable but non-culturable state in foodborne pathogens. *Frontiers in Microbiology*, 8(580): 1-16.

Zolfaghari, M, Rezaei, M, Mohabbati Mobarez, A, Forozandeh Moghaddam, M, Hosseini, H & Khezri, M 2020, Virulence genes expression in viable but non-culturable state of *Listeria monocytogenes* in fish meat. *Food Science and Technology International*, 26: 205-12.

Bibliographic information of this paper for citing:

Khezri, M, Rezaei, M, Mohabbati Mobarez, A, Zolfaghari, M 2021, The change in heat inactivation of *Escherichia coli* O157:H7 after entering into the viable but non-culturable state in salted fish, *Hypophthalmichthys molitrix*. Caspian Journal of Environmental Sciences, 19: 629-637