

## ORIGINAL ARTICLE/ORIJİNAL ÇALIŞMA

FULL PAPER

TAM MAKALE

## THE EFFECT OF WASHING AND COOKING ON RESIDUE LEVELS OF SHRIMP TREATED WITH 4-HEXYLRESORCINOL

**Arif Selçuk<sup>1</sup>, Özkan Özden<sup>2</sup>**<sup>1</sup> TÜBİTAK Marmara Research Center, Food Institute, Gebze/ Kocaeli- Turkey<sup>2</sup> Istanbul University, Faculty of Fisheries, Department of Seafood Processing Technology and Safety, Istanbul-Turkey

Received: 09.09.2016

Accepted: 14.11.2016

Published online: 30.12.2016

**Corresponding author:****Özkan ÖZDEN**, Istanbul University, Faculty of Fisheries, Department of Seafood Processing Technology and Safety, Ordu Caddesi No:8, 34134 Laleli Fatih, Istanbul-TurkeyE-mail: [ozden@istanbul.edu.tr](mailto:ozden@istanbul.edu.tr)**Abstract:**

The aim of this study was to investigate the effects of washing with tap water and different cooking process on residue levels of shrimp treated with 4-hexylresorcinol. Dipping solutions of 4-hexylresorcinol in five different concentrations (5-10-50-100-500 ppm) were prepared using distilled water. The shrimps were dipped into the solutions for 1-5-10- 30 min. Half of the samples were washed with tap water. The residue level of 4-hexylresorcinol in all samples were analysed after frying and boiling process. The 4-hexylresorcinol residual levels in unwashed and washed raw, fried and boiled shrimp samples showed linear increases with increasing dipping time and concentration. The best results were obtained for the treatment of 5 and 10 ppm for 1-5-10-30 min.

**Keywords:** Shrimp, *Parapenaeus longirostris*, Black-spot, Melanosis, 4-hexylresorcinol

## Introduction

The demand to fishery products is increasing each passing day because of global warming and inefficiencies in agricultural production. Capita fish consumption per person in the world weight 1960s 9.9 kg, while increased in year 2012 to 19.2 kg. Fish and fishery products play a critical role in global food security and nutritional needs of people in all world countries. In 2012, capture production of shrimp species registered a new maximum at 3.4 million tonnes and shrimp as a fishing product has one of the highest economic value of world (FAO 2014).

Deepwater pink shrimp (*Parapenaeus longirostris*) is economically important fishery resources in Spain, France, Italy, Greece, Sicily and Tunisia, all of the East Atlantic and Mediterranean and Marmara Sea.

Fisheries products help the consumer by life maintain good health with all essential nutrients components. Shrimp is delicious seafood's and important sources of protein, low in saturated fat, vitamin B12, selenium,  $\omega$ -3 highly unsaturated fatty acids (HUFA) and astaxanthin, a potent natural antioxidant for human consumption worldwide (Heu et al, 2003; Dayal Syama et al., 2013). However, this high value nutritional components shrimp is perishable due to enzymatic and microbiological spoilage during post-mortem storage (Nirmal and Benjakul 2010). Melanosis or black spots occurs in shrimp (Figure 1.), lobster and others Crustaceans during storage as a result of the action of polyphenol oxidase (PPO) on tyrosine or its derivatives (Martínez-Alvarez *et al.*, 2009). The active PPO catalyses the hydroxylation to benzoquinones of o-dihydroxyphenols. Benzoquinones react non-enzymatically with a variety of compounds, like amines, amino acids, or O<sub>2</sub>, forming melanin's, responsible for black coloration during storage (Nirmal & Benjakul, 2010; Zamorano *et al.*, 2009).

The appearance of melanosis during storage drastically reduces their market value, nutritional value and consumer acceptability (Martínez-Alvarez *et al.*, 2005; Mayer, 2006; Erkan *et al.*, 2007). The refrigeration of shrimps or their storage on ice can slow down this process, but not inactivate it. The presence of black spots makes necessary the

use of antimelanotics. Therefore, addition of chemicals is necessary to prevent melanosis in shrimps. Many studies have focused on the prevention of browning and microbial spoilage of shrimp by naturally occurring and synthetic tyrosinase inhibitors (Martínez-Alvarez *et al.*, 2007; Nirmal & Benjakul 2011a; 2011b). Sodium metabisulphite is the chemical most widely used to control the melanosis of shrimps during the storage. It acts by inactivating the enzyme polyphenoloxidase and combining with quinones to prevent their polymerization in pigmented compounds. Nonetheless, the treatment of food products with sodium metabisulphite can caused anaphylactic reactions to sulphite-sensitive individuals and bronchoconstriction to asthmatic patients. Moreover, this chemical produces an alkaline pollution of the environment that kills several aquatic species. The use of alternative compounds is necessary and is reported as good alternative 4-hexylresorcinol (Guandalini *et al.*, 1998; Montero *et al.*, 2001, 2004, 2005; Thepnuan *et al.*, 2008). This compound is considered GRAS (Generally Recognized As Safe) in many countries (Australia, Brazil, Canada, USA, etc.). 4-hexylresorcinol approved as a food additive (E586) as defined by Council Directive 89/107/EEC article 5 amending Directive 95/2/EC. Fresh, frozen and deep-frozen crustaceans to a maximum residue level of 2 mg/kg in crustacean meat. A single EC Regulation 1333/2008 on food additives has been adopted intended to replace and repeat Directives 89/107/EEC and 95/2/EC. According to Directive 2003/89/EC, 4-hexylresorcinol is not subject to allergen labelling. However, it is up to the discretion of each individual country to adopt labelling measures. According to Canada Food and Drug Regulation 1078 (1998), the residues of 4-hexylresorcinol in the edible portion of the uncooked product not exceed 1.0 mg/kg. According to China Food Additives Hygiene Standard (GB 2760-1996), 4-hexylresorcinol were classified as antioxidant. Residue level for the prevent of browning in shrimp were reported as  $\leq 1$  mg/kg (1ppm). Acceptable Daily Intake (ADI) "treatment of crustacean at concentrations of up to 50 mg/L, resulting in residue levels of approximately 1 mg/kg in edible portion, is not of toxicological concern" (JECFA, 1995).

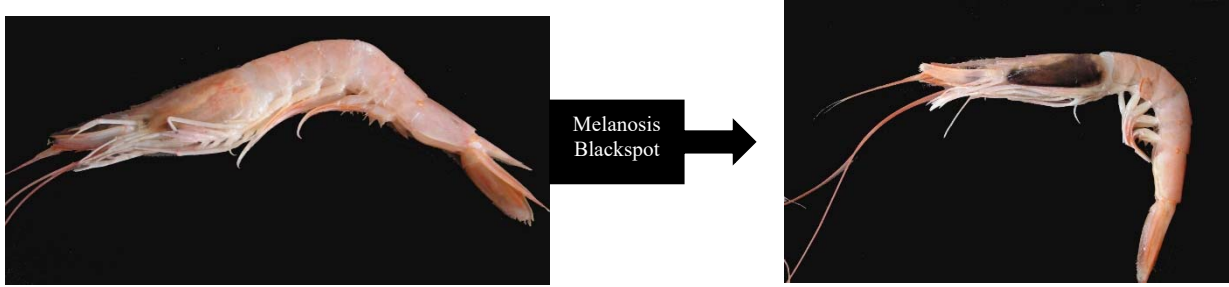


Figure 1. Malanosis/Blackspot in shrimp (Foto: Özkan Özden)

In this study were investigated the residue levels of shrimp treated with 4-hexylresorcinol in different concentration/dipping time and the effect of washing with tap water and frying and boiling process in residue levels after dipping treatment.

**Materials and Methods**

Samples of deep-water pink shrimp (*Parapenaeus longirostris* Lucas, 1846) was caught in the Marmara Sea (Tuzla Kayikhanesi-Istanbul) by a commercial fishing trawler. On board, the shrimp was washed with seawater and were transported with ice (shrimp/ice = 1/1 w/w) to the laboratory. Samples of deep-water pink shrimp (2500 g for each group) were dipped for 1, 5, 10, 30 min in cold tap water (7°C) solutions of 5, 10, 50, 100, 500 ppm 4-hexylresorcinol. Afterwards the shrimp was removed, each group samples divided two group.

First groups were washed with tap water in 15 second, other groups non washed. Washed an unwashed shrimp samples were peeled. Samples for each groups were divided three lot. The first group of shrimp samples were not cooked. Second and third lot samples were cooked by frying (in sun flower oil at 180°C 3 min) and boiling (in boiling water for 3 min). The process was repeated in new captured shrimp samples after two days. Total 240 group samples (5 concentration (5-10-50-100-500 ppm) x 4 dipping time (1-5-10- 30 min) x first processing (unwashed and washed) x second processing (raw- frying - boiling) = 5x4x2x3=120 x 2=240 samples)) were also homogenized and stored in sterile plastic sample containers at -20°C until analysis of residues. 4-Hexylresorcinol analysis in each group sample was performed in three duplicate. The results are presented as the mean of the three measurements for each group (Figure 2).

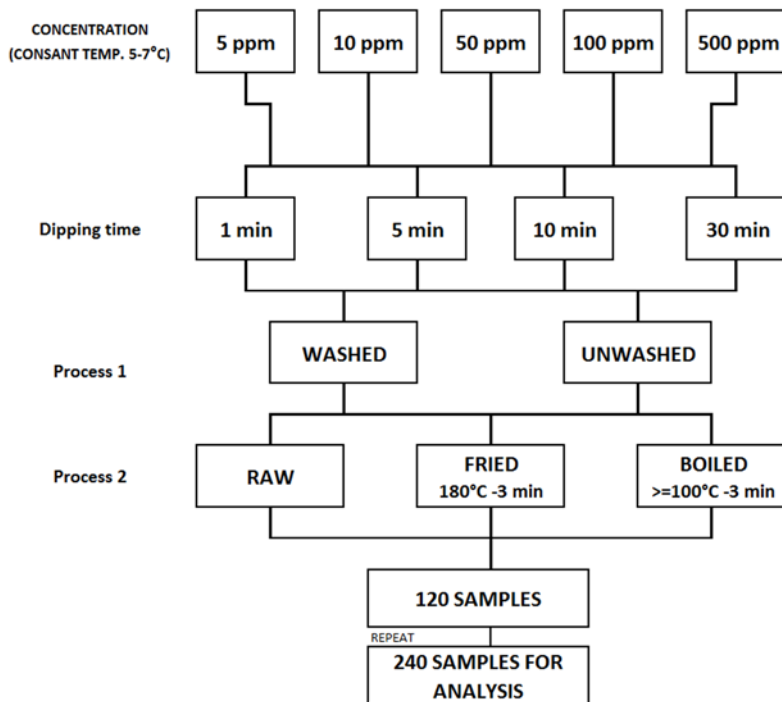


Figure 2. Full factorial experimental design for residue of 4-Hexylresorcinol in shrimp

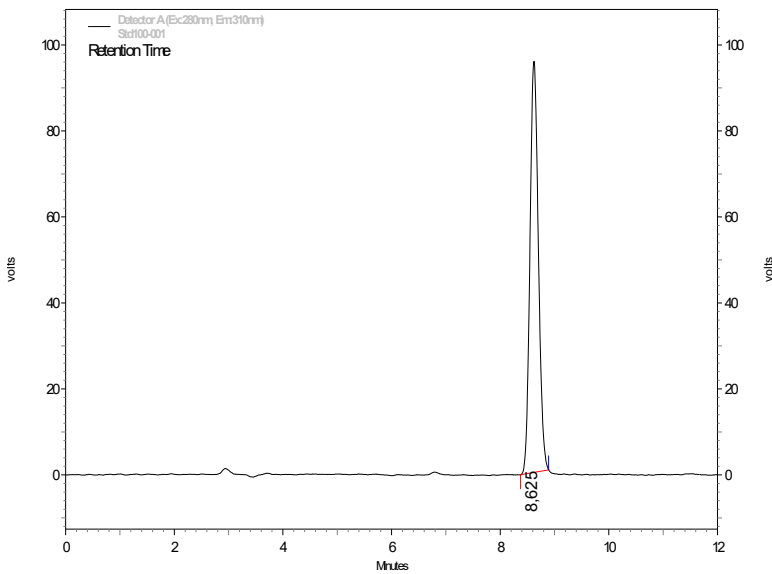
The samples were analysed by high-performance liquid chromatography (HPLC) (Shimadzu LC 10 AT Vp series pump, Shimadzu SIL 10AD Vp cooling automatic sampling (4°C), Shimadzu RF 10AXL fluorescence detector (FLD), Shimadzu CTO 10AV Vp, Shimadzu SCL 10A Vp, Class-Vp 6.14 - Japan) with a fluorescence detector, with the described method by Selçuk and Özden (2014a, 2014b). The concentration of 4-hexylresorcinol was measured by comparing its retention

time with those of authentic standards (Acros Organics #197920250: 4-hexylresorcinol, 99%), and the 4-hexylresorcinol content was calculated on a weight basis:

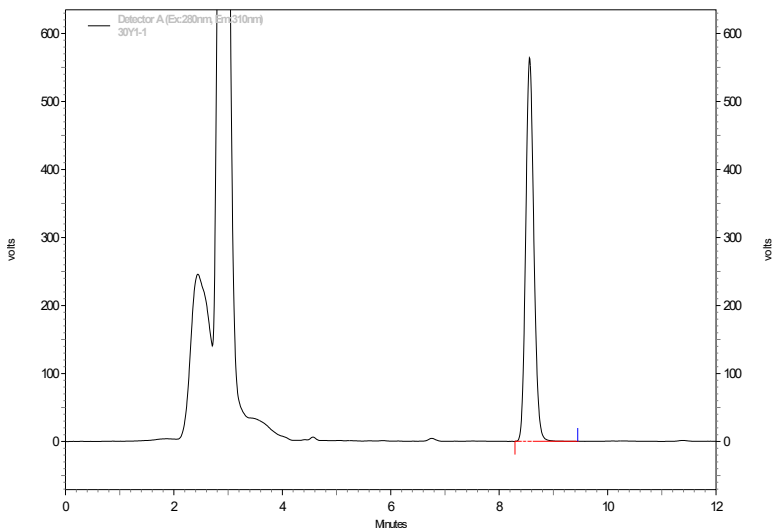
$$4\text{-hexylresorcinol (mg/kg)} = \text{HPLC value read } (\mu\text{g/L}) \times 0.250 \text{ L} / \text{sample weight (g)} \text{ (Figure 3.)}$$

**Statistical analysis**

Statistical analysis of the data was performed in STATISTICA 7 software (StatSoft Tulsa-USA), with ANOVA post-hoc test ( $p \leq 0.05$ ).



**A. 4-hexylresorcinol Standard**



**B. 4-hexylresorcinol in Shrimp**

**Figure 3.** 4-hexylresorcinol HPLC chromatograms

## Results and Discussion

In this study, the residue level in all samples showed linear increase with dipping time and concentration of dipping solutions. The residual levels of the samples treated with 4-hexylresorcinol are presented in Table 1. The residue levels of washed raw, fried and boiled samples were found lower ( $p < 0.05$ ) compared to unwashed samples in all concentrations and dipping times. According to, The Scientific Committee of Food of the European Commission and the proposal of the EU Commission were reported 2 ppm as the maximum limit for the 4-hexylresorcinol residue in the consumable portion of crustaceans (Directive 95/2/EC). The residue level in legal limit were found in shrimp samples treated with 5-10 ppm of 4-hexylresorcinol for 1-5-10-30 min and 50 ppm of 4-hexylresorcinol for 1-5 min. Additionally, the residue level in 50 ppm 4-hexylresorcinol for 10 min treated washed raw, fried and boiled shrimp samples were found below 2 ppm. The 1 ppm legal limit established for 4-hexylresorcinol residue level in Canada Food and Drug Regulation, and China Food Additives Hygiene Standard. Considering these values, in this study were found as safe treatment with 5-10 ppm of 4-hexylresorcinol for 1-5-10-30 min for unwashed shrimp samples and up to 50 ppm 1 min for washed shrimp samples. Our results showed similar with earlier scientific studies. Iyengar *et al.* (1991) found that the residue

level increased with the increment in the dipping period. They detected the residue level as 0.6 mg/kg in the shrimp samples which were dipped in % 0.005 4-Hexylresorcinol solution for 1 minute. They also observed that the residue level rised to 1.0 mg/kg when they increased the dipping period to 15 minutes. Guandalini *et al.* (1998) found the residue level as 20, 42 ve 85 mg/kg in pink shrimps which were subjected to 25, 50 and 100 ppm (mg/kg) concentration of 4-Hexylresorcinol solution. Montero *et al.* (2005) reported the residue level as 2.73 ppm, 3.22 ppm, 2.57 ppm and 5.91 ppm in the 0.01% concentration of 4-Hexylresorcinol in the 2th, 4th, 7th and 5th day respectively. It was stated that the use of 4-Hexylresorcinol (0.1 % and 0.05 %) solutions can be a good alternative to commercial sulphite based products in order to prevent melanosis in Norway lobster. Besides it was concluded in the same study that the concentration of 0.05 % can prevent melanosis for 12 days (Martinez-Alvarez *et al.*, 2007). The deep-water pink shrimp samples were dipped in 0.1% 4-Hexylresorcinol solution for 3, 30 and 60 minutes and were found the level of residue in the samples as 5.5, 5.2 and 7.2 mg/kg, respectively. In the same study, after dipped in 0.25% 4-Hexylresorcinol solution for 60 minute, the level of residue was detected as 14.3, 19.3 and 22.0 mg/kg. After the washing of samples in 5 liter water for 2 minutes, the level of residue showed an increase (Mendes *et al.*, 2006).

**Table 1.** The level of 4-hexylresorcinol residues in deep-water shrimps (ppm in wet weight).

Concentration	Dipping		Unwashed Raw	Washed Raw	Unwashed Fried	Washed Fried	Unwashed Boiled	Washed Boiled
	Time							
5 ppm	1 min		0.08 ± 0.01 <sup>Aa</sup>	0.05 ± 0.00 <sup>Ab</sup>	0.09 ± 0.01 <sup>Aa</sup>	0.06 ± 0.01 <sup>Ab</sup>	0.07 ± 0.00 <sup>Aa</sup>	0.06 ± 0.01 <sup>Ab</sup>
	5 min		0.15 ± 0.01 <sup>Ba</sup>	0.12 ± 0.02 <sup>Ba</sup>	0.13 ± 0.01 <sup>Ba</sup>	0.12 ± 0.01 <sup>Ba</sup>	0.12 ± 0.01 <sup>Ba</sup>	0.09 ± 0.01 <sup>Bb</sup>
	10 min		0.26 ± 0.00 <sup>Ca</sup>	0.20 ± 0.02 <sup>Cb</sup>	0.23 ± 0.01 <sup>Ca</sup>	0.19 ± 0.01 <sup>Cb</sup>	0.19 ± 0.01 <sup>Ca</sup>	0.15 ± 0.01 <sup>Cb</sup>
10 ppm	30 min		0.32 ± 0.03 <sup>Da</sup>	0.28 ± 0.01 <sup>Db</sup>	0.32 ± 0.01 <sup>Da</sup>	0.25 ± 0.01 <sup>Db</sup>	0.32 ± 0.01 <sup>Da</sup>	0.24 ± 0.01 <sup>Db</sup>
	1 min		0.22 ± 0.02 <sup>Ea</sup>	0.18 ± 0.05 <sup>Ca</sup>	0.19 ± 0.01 <sup>Ea</sup>	0.11 ± 0.02 <sup>Bb</sup>	0.15 ± 0.01 <sup>Ba</sup>	0.12 ± 0.00 <sup>Eb</sup>
	5 min		0.29 ± 0.02 <sup>Fa</sup>	0.25 ± 0.01 <sup>Eb</sup>	0.24 ± 0.02 <sup>Ca</sup>	0.20 ± 0.01 <sup>Cb</sup>	0.25 ± 0.03 <sup>Ea</sup>	0.20 ± 0.00 <sup>Fb</sup>
50 ppm	10 min		0.45 ± 0.03 <sup>Ga</sup>	0.36 ± 0.03 <sup>Fb</sup>	0.42 ± 0.01 <sup>Fa</sup>	0.31 ± 0.00 <sup>Eb</sup>	0.32 ± 0.01 <sup>Fa</sup>	0.28 ± 0.00 <sup>Gb</sup>
	30 min		0.75 ± 0.10 <sup>Ha</sup>	0.60 ± 0.05 <sup>Gb</sup>	0.55 ± 0.06 <sup>Ga</sup>	0.49 ± 0.01 <sup>Fa</sup>	0.59 ± 0.02 <sup>Ga</sup>	0.48 ± 0.00 <sup>Hb</sup>
	1 min		<b>1.15 ± 0.22<sup>Ia</sup></b>	0.84 ± 0.09 <sup>Hb</sup>	0.95 ± 0.08 <sup>Ha</sup>	0.67 ± 0.10 <sup>Gb</sup>	<b>1.01 ± 0.03<sup>Ha</sup></b>	0.71 ± 0.03 <sup>Ib</sup>
100 ppm	5 min		<b>1.66 ± 0.06<sup>Ja</sup></b>	<b>1.34 ± 0.12<sup>Ib</sup></b>	<b>1.46 ± 0.06<sup>Ja</sup></b>	<b>1.24 ± 0.03<sup>Hb</sup></b>	<b>1.81 ± 0.06<sup>Ja</sup></b>	<b>1.49 ± 0.05<sup>Ib</sup></b>
	10 min		<b>2.12 ± 0.13<sup>Ka</sup></b>	<b>1.76 ± 0.12<sup>Ib</sup></b>	<b>1.96 ± 0.07<sup>Ja</sup></b>	<b>1.69 ± 0.12<sup>Ib</sup></b>	<b>2.27 ± 0.11<sup>Ja</sup></b>	<b>1.73 ± 0.06<sup>Kb</sup></b>
	30 min		<b>2.93 ± 0.04<sup>La</sup></b>	<b>1.64 ± 0.25<sup>IJb</sup></b>	<b>3.37 ± 0.08<sup>Ka</sup></b>	<b>2.43 ± 0.10<sup>Ib</sup></b>	<b>3.97 ± 0.15<sup>Ka</sup></b>	<b>2.85 ± 0.00<sup>Lb</sup></b>
500 ppm	1 min		<b>2.33 ± 0.03<sup>Ma</sup></b>	<b>2.69 ± 0.20<sup>Kb</sup></b>	<b>1.97 ± 0.03<sup>Ja</sup></b>	1.43 ± 0.25 <sup>Ib</sup>	<b>2.06 ± 0.07<sup>Ja</sup></b>	1.85 ± 0.03 <sup>Mb</sup>
	5 min		<b>2.77 ± 0.12<sup>NLa</sup></b>	<b>2.64 ± 0.07<sup>Ka</sup></b>	<b>2.76 ± 0.29<sup>La</sup></b>	<b>2.24 ± 0.17<sup>Ib</sup></b>	<b>2.77 ± 0.08<sup>La</sup></b>	<b>2.05 ± 0.07<sup>Nb</sup></b>
	10 min		<b>3.89 ± 0.05<sup>Oa</sup></b>	<b>3.08 ± 0.35<sup>Lb</sup></b>	<b>3.68 ± 0.08<sup>Ma</sup></b>	<b>3.30 ± 0.25<sup>Kb</sup></b>	<b>3.82 ± 0.26<sup>Ka</sup></b>	<b>3.05 ± 0.08<sup>Ob</sup></b>
500 ppm	30 min		<b>4.87 ± 0.11<sup>Öa</sup></b>	<b>4.22 ± 0.14<sup>Mb</sup></b>	<b>7.01 ± 0.41<sup>Na</sup></b>	<b>4.78 ± 0.66<sup>Lb</sup></b>	<b>6.57 ± 0.21<sup>Ma</sup></b>	<b>5.03 ± 0.34<sup>Öb</sup></b>
	1 min		<b>15.90 ± 0.89<sup>Pa</sup></b>	<b>12.48 ± 0.42<sup>Nb</sup></b>	<b>11.46 ± 0.48<sup>Oa</sup></b>	<b>9.78 ± 1.02<sup>Mb</sup></b>	<b>13.81 ± 0.29<sup>Na</sup></b>	<b>9.69 ± 0.16<sup>Pb</sup></b>
	5 min		<b>20.86 ± 0.19<sup>Ra</sup></b>	<b>19.01 ± 0.96<sup>Ob</sup></b>	<b>17.14 ± 0.71<sup>Öa</sup></b>	<b>15.10 ± 0.53<sup>Nb</sup></b>	<b>19.34 ± 0.03<sup>Oa</sup></b>	<b>16.13 ± 0.32<sup>Pb</sup></b>
500 ppm	10 min		<b>24.51 ± 0.14<sup>Sa</sup></b>	<b>22.38 ± 0.37<sup>Öb</sup></b>	<b>25.46 ± 0.30<sup>Pa</sup></b>	<b>20.93 ± 2.35<sup>Ob</sup></b>	<b>25.34 ± 0.49<sup>Öa</sup></b>	<b>23.08 ± 0.38<sup>Sb</sup></b>
	30 min		<b>35.02 ± 4.21<sup>Ta</sup></b>	<b>27.10 ± 1.79<sup>Pb</sup></b>	<b>29.35 ± 0.43<sup>Ra</sup></b>	<b>27.63 ± 1.11<sup>Pb</sup></b>	<b>34.26 ± 1.27<sup>Pa</sup></b>	<b>30.03 ± 1.71<sup>Tb</sup></b>

**Bold represents unacceptable residue levels for EU and China (grey line)**

All values are the mean ± standard deviation (n = 3)-Different letters (A,B,C) in the same column indicate significant differences ( $p < 0.05$ )-Different letters (a,b,c) in the same line indicate significant differences ( $p < 0.05$ )



## Conclusion

In all concentrations and dipping times, the residue levels of washed (raw, fried and boiled) shrimps were significantly lower ( $P < 0.05$ ) than those for unwashed shrimp samples. The legal limit (for EU and China regulation) for residue level not exceeded in shrimp samples treated with 5-10 ppm of 4-hexylresorcinol for 1-5-10-30 min and 50 ppm of 4-hexylresorcinol for 1 min only in washed shrimps.

## Acknowledgements

This work was supported by the Research Found of the University of Istanbul. Project number: 4256.

## References

- Erkan, N., Özden, Ö., Üçok Alakavuk, D., Tosun, Y.Ş., Varlık, C. & Baygar, T. (2007). İstanbul'da Satılan Karideslerin Sodyum Metabisülfid Düzeyinin Tespiti. *Journal of Fisheries Sciences.com*, 1(1), 26-33.
- Dayal Syama, J., Ponniah, A.G., Imran Khan, H., Madhu Babu, E.P., Ambasankar, K. & Kumarguru Vasagam, K.P. (2013). Shrimps – a Nutritional Perspective. *Current Science*, 104(11), 1487-1491.
- FAO (2014). The State of World Fisheries and Aquaculture, Opportunities and challenges. Rome 2014 e-ISBN 978-92-5-108276-8 <http://www.fao.org/3/a-i3720e.pdf> (accessed 10/03/2016)
- Food and Drug Regulations 1078 (1998). Regulations Amending the Food and Drug Regulations (1078), 1998-1599 15 September, 1998. <http://publications.gc.ca/gazette/archives/p2/1998/1998-09-30/pdf/g2-13220.pdf> (accessed 10/03/2016)
- GB 2760-1996 (1996). Food Additives Hygiene Standard. Published by Ministry of Public Health, PR China.
- Guandalini, E., Ioppolo, A., Mantovani, A., Stacchini, P. & Giovannini, C. (1998). 4-hexylresorcinol as Inhibitor of Shrimp Melanosis: Efficacy and Residues Studies; Evaluation of Possible Toxic Effect in a Human Intestinal in Vitro Model (caco-2); Preliminary Safety Assessment. *Food Addit Contam Part A.*, 15(2), 171-180.
- Heu, M.S., Kim, J.S., Shahidi, F. (2003). Components and nutritional quality of shrimp processing by-products. *Food Chemistry*, 82, 235-242.
- Iyengar, R., Bohmont, C.W. & Mc Evily, A.J. (1991). 4-Hexylresorcinol and Prevention of Shrimp Blackspot: Residual Analyses. *Journal of Food Composition and Analysis*, 4, 148-157.
- JECFA (1995). Evaluation of Certain Food Additives and Contaminat. 44th FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, 859, pp. 1-53.
- Nirmal, P.N. & Benjakul, S. (2010). Effect of Catechin and Ferulic Acid on Melanosis and Quality of Pacific White Shrimp Subjected to Prior Freeze-Thawing During Refrigerated Storage. *Food Control*, 21(9), 1263-1271.
- Nirmal, P.N. & Benjakul, S. (2011a). Use of Tea Extracts for Inhibition of Polyphenoloxidase and Retardation of Quality Loss of Pacific White Shrimp During Iced Storage. *LWT-Food Science and Technology*, 44(4), 924-932.
- Nirmal, P.N. & Benjakul, S. (2011b). Inhibition of Melanosis Formation in Pacific White Shrimp by the Extract of Lead (*Leucaena leucocephala*) Seed. *Food Chemistry*, 128(2), 427-432.
- Martínez-Alvarez, O., Montero, P. & Gómez-Guillén, M.C. (2005). Controlled Atmosphere as Coadjuvant to Chilled Storage for Prevention of Melanosis in Shrimps (*Parapenaeus longirostris*). *European Food Research and Technology*, 220, 125-130.
- Martínez-Alvarez, O., López-Caballero, M.E., Montero, P. & Gómez-Guillén, M.C. (2007). Spraying of 4-hexylresorcinol Based Formulations to Prevent Enzymatic Browning in Norway Lobsters (*Nephrops norvegicus*) During Chilled Storage. *Food Chemistry*, 100, 147-155.
- Mayer, A.M. (2006). Polyphenol Oxidases in Plants and Fungi: Going places? A review. *Phytochemistry*, 67, 2318-2331.
- Mendes, R., Pestana, J. & Pestana, C. (2006). Changes in 4-hexylresorcinol Residues During Processing of Deepwater Pink

- Shrimp (*Parapenaeus longirostris*). *European Food Research and Technology*, 223, 509-515.
- Montero, P., López-Caballero, M.E. & Pérez-Mateos, M. (2001). The Effect of Inhibitors and High Pressure Treatment to Prevent Melanosis and Microbial Growth on Chilled Prawns (*Penaeus japonicus*). *Journal of Food Science*, 66, 1201-1206.
- Montero, P., Martínez-Alvarez, O. & Gómez-Guillén, M.C. (2004). Effectiveness of Onboard Application of 4-Hexylresorcinol in Inhibiting Melanosis in Shrimp (*Parapenaeus longirostris*). *Journal of Food Science*, 69, C643-C647.
- Montero, P., Martínez-Alvarez, O., Gómez-Guillén, M.C., Zamorano, J.P. & Alique, R. (2005). Melanosis Inhibition and 4-hexylresorcinol Residual Levels in Deepwater Pink Shrimp (*P. longirostris*) Following Various Treatments. *European Food Research and Technology*, 223(1), 16-21.
- Regulation (EC) No 1333/2008 (2008). Regulation (EC) no 1333/2008 of the European parliament and of the council of 16 December 2008 on food additives. Official Journal of the European Union, L354/16-L354/33.
- Thepnuan, R., Benjakul, S. & Visessanguan, W. (2008). Effect of Pyrophosphate and 4-hexylresorcinol Pretreatment on Quality of Refrigerated White Shrimp (*Litopenaeus vannamei*) Kept under Modified Atmosphere Packaging. *Journal of Food Science*, 73(3), S124-S133.
- Selçuk, A. & Özden, Ö. (2014a). A Rapid HPLC Method for Determination of 4-hexylresorcinol Residues in Shrimp. *Journal of Fisheries Sciences.com*, 8(2), 131-138.
- Selçuk, A. & Özden, Ö. (2014b). Mathematical Modelling of 4-hexylresorcinol Residue to Ensure Consumer Safety. *Quality Assurance and Safety of Crops & Foods*, 6(4), 425-430.
- Zamorano, J.P., Martínez-Alvarez, O., Montero, P., Gómez-Guillén, M.C. (2009). Characterisation and Tissue Distribution of Polyphenol Oxidase of Deepwater Pink Shrimp (*Parapenaeus longirostris*). *Food Chemistry*, 112,104-111.
- 89/107/EEC (1989). Council Directive of 21 December 1988 on the approximation of the laws of the Member States concerning food additives authorized for use in foodstuffs intended for human consumption. 1989L0107 — EN — 10.09.1994 — 001.001 — 1-10.
- 95/2/EC (1995). European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. 1-53.
- 2003/89/EC (2003). Directive 2003/89/EC of the European parliament and of the council of 10 November 2003 amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs. Official Journal of the European Union, L308/15-L308/18.