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Mathematical modelling of 4-hexylresorcinol residue to ensure consumer safety

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RESEARCH ARTICLE

Abstract

Shrimp is a major source of animal protein and is of great economic importance in world markets. The occurrence of black spot in shrimp after harvesting is a major problem for the aquaculture and fisheries industries. 4-hexylresorcinol has recently been approved by the European Union as an alternative chemical for the prevention of black spot in shrimp. However, high residual levels of 4-hexylresorcinol have important negative effects on humans. The correct mathematical design of 4-hexylresorcinol residue analyses should ensure the cost-effective operation of these industries, their environmental sensitivity, and safe food management practices. The mathematical determination of 4-hexylresorcinol residues will circumvent the high cost of high-performance liquid chromatographic analyses, ensuring high shrimp quality and adherence to food safety standards.

Keywords: 4-hexylresorcinol, mathematical determination, residue level, shrimp

1. Introduction

Food products may contain unwanted pesticides and veterinary medicine residues, mycotoxins and other natural toxins, unapproved or excess amounts of additives, pollutants from environment and unsuitable ingredients. Additives are used for colour protection and nutritional reasons but they may have unexpected effects which were not foreseen (Thomson *et al.*, 2012).

Laboratory analyses are important tools in food safety in terms of verification and approval. Chromatography and spectrophotometers are typical techniques that are used. Generally, utilisation of these tools requires well trained operators and high tech equipment's so they are not practically implemented in food processing workplaces (Lebesi *et al.*, 2010). Globalization and consumer requests demands new processing and distribution strategies deriving from new food safety risks and difficulties (Poms, 2013).

Shrimp is one of the most important crustacean species naturally distributed in fishing catchment areas worldwide. Because of its nutritional and curative properties, shrimp has become one of the most valuable seafoods in the world. Since 2011, the total annual global catch of shrimp has exceeded 6,519,671 tonnes (FAO, 2011). The greatest problem in the production of shrimp is the formation of melanosis (black spot), which causes financial losses for fishermen. Therefore, fishermen use a variety of chemicals to prevent melanosis to minimise their profit losses.

Melanosis is the darkening of pigments in the shrimp membranes and meat just beneath the shell, which causes a visual defect that compromises the shrimp's marketability. Polyphenol oxidase acts on naturally occurring colourless phenols, producing coloured quinines in the shrimp. These quinines then polymerise to form dark insoluble melanin (Mendes *et al.*, 2006). Melanosis will eventually occur even at ideal refrigeration temperatures, but two chemicals are commonly used to delay its onset. The most widely used is sodium metabisulphite, a relatively inexpensive inorganic chemical that has been used for more than 40 years (Miget, 2010). The shrimp are usually dusted with commercial sulphite-based products while still on board the fishing vessel, regardless of whether they are sold as fresh or cooked. Sulphites can induce specific adverse reactions

in some population groups, especially asthmatics, and for that reason, 4-hexylresorcinol has recently been approved by the European Union (EU) as an alternative chemical for the prevention of melanosis. The European Scientific Committee on Food considers 4-hexylresorcinol to be toxicologically acceptable for the prevention of melanosis in shrimp under the conditions described, provided the residues in the crustacean meat do not exceed 2 mg/kg (EC, 2003b). The other maximum acceptable residue levels for 4-hexylresorcinol given in Table 1.

4-hexylresorcinol is widely used as an antiseptic in different contexts, including in antimicrobial soaps, personal health-care hand washes, preoperative skin preparations, antiseptic skin and wound cleansers, mouthwashes, and cold and cough preparations. According to National Toxicology Program (1988), the administration of 4-hexylresorcinol is associated with three types of tumours: mononuclear cell leukaemia in male and female rats, hepatocellular neoplasms in male mice, and circulatory system tumours in male and female mice. However, most studies have reported that it is harmless.

Improvements to the mathematical models used to simulate 4-hexylresorcinol residues and their adaptation to specific applications are important in maintaining food safety (An *et al*., 2002; Penycook *et al*., 2004; Pringer, 2007; Veraart and Coulier, 2007).

During the past decade, the use of this chemical to prevent melanosis in shrimp has increased in many countries. Although legal restrictions apply, serious violations have been reported, with concomitant problems. This study provides a mathematical simulation formula to allow the sensitive determination of 4-hexylresorcinol residues for use by fish and shrimp traders.

2. Materials and methods

Samples

Fresh deep-water pink shrimp (*Parapenaeus longirostris,* Lucas, 1846) were caught in the Marmara Sea in the winter of 2009. Samples of 50 kg were used for the experiment. The shrimp were packed with ice in separate insulated polystyrene boxes (approx. 10 kg per box) and delivered to the laboratory within 2 h of capture. All individuals were from a single catch. The air temperature at the time of capture was 6-10 °C.

Model design

The samples were divided into groups according to the experimental design. A 3^2 full-factorial design was used to study the effects of the dipping solution concentration (C) and the time of exposure (t) on the level of 4-hexylresorcinol residue in the shrimp. In this design, two factors were evaluated, each at three levels (5, 50, and 100 mg/kg × 1, 5, and 10 min), and the experimental trials were performed with all nine possible combinations (Figure 1). In this experiment, the full-factorial design was used with the response surface regression test in the Statistica 7 software (StatSoft Inc., Tulsa, OK, USA). A response-surface chart was constructed with the same programme.

Table 1. Maximum acceptable residue levels of 4-hexylresorcinol (classified as E586, CAS 136-776) in the world.

Legislation	Maximum acceptable levels
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 (EC, 1989, 1995)
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 (EC, 1989, 2003a, 1995, 2008)
Food Standards Australia New Zealand (FSANZ) standard 1.3.1 of the Code for Food Additives	good manufacturing practice (FSANZ, 2013) (lowest level possible of an additive to achieve a technological function)
Food and Drug Regulation 1078 (1998), Canada	good manufacturing practice; residues in the edible portion of the uncooked product not to exceed 1.0 mg/kg
Food Additives Hygiene Standard (GB 2760-1996); classified as an antioxidant (04.013), China PR	to prevent shrimps from browning; residue level ≤1 mg/kg (Ministry of Public Health of the People's Republic of China, 1996)
Joint FAO/WHO Expert Committee on Food Additives (JECFA)	ADI 'treatment of *Crustacea* 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)

ADI = acceptable daily intake

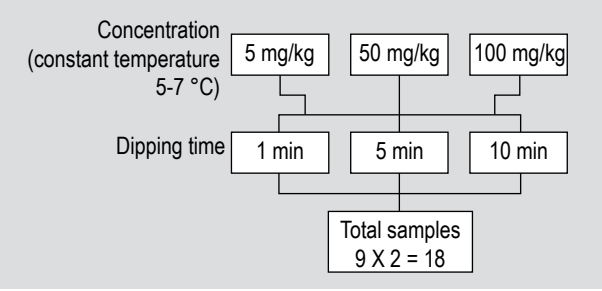

Figure 1. Experimental design.

Determination of 4-hexylresorcinol residues

The model results were validated by analyses of the same samples by high-performance liquid chromatography (HPLC) with a fluorescence detector, according to the method of Jonker and Dekker (2000), with some modification as described in Selçuk and Özden (in press).

In short, 10 ml deionised water was added to 5±0.01 g of homogenised shrimp muscle in 50 ml centrifuge tubes and the tubes were shaken for 30 s to disperse the shrimp meat in the water. 15 ml methanol was then added and the tissue homogenised with an Ultra-Turrax T25 homogeniser (Janke and Kunkel, IKA Labortechnik, Staufen, Germany). The sample was centrifuged (Hettich Universal 320R; Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany) at 3.000×g for 5 min. Supernatant was separated into a 50 ml volumetric flask. The extraction procedure was repeated twice (each time with 10 ml methanol), the extracts were combined and than diluted to volume. An aliquot of the extract (5 ml) was transferred to a 25 ml volumetric flask, an equal volume of the mobile phase was added, and the sample was filtered through a nylon membrane filter (pore size 0.22 µm, filter size 13 mm; E-Chrom Tech, Taipei, Taiwan) (Table 2).

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 (mg/kg) was calculated on a weight basis:

$$\text{4-hexylresorcinol} = \frac{\text{HPLC value read} \times 0.250}{\text{sample weight}} \quad (1)$$

With HPLC value read in µg/l and sample weight in g.

Statistical analysis

The data are presented as arithmetic means for each experimental point. The means were compared using Statistica 7 (StatSoft Inc.) and differences were considered significant at $P \leq 0.05$ on an analysis of variance post hoc test.

3. Results

According to the regression analysis, the concentration × time interaction had a statistically significant effect ($P<0.05$) on the amount of 4-hexylresorcinol residue. Of the alternative factors upon which the model was based, the linear and quadratic effects of time (in minutes) on the amount of 4-hexylresorcinol residue were insignificant compared with the effects of the other model parameters. At the lowest 4-hexylresorcinol concentration, an increase in the treatment time did not significantly increase the amount of 4-hexylresorcinol residue. However, at the highest 4-hexylresorcinol concentration, the amount of 4-hexylresorcinol residue was significantly dependent on the exposure time ($P<0.05$). Similarly, the increase in the amount of 4-hexylresorcinol residue that occurred at the higher concentrations accelerated as the treatment time increased.

With Equation 2, which was established by evaluating the results of a three-dimensional response-surface chart constructed with the Statistica programme, no retreatment or further experimentation is required to calculate the amount of residue produced in an industrial context.

$$\text{4-hexylresorcinol residue level (mg/kg)} = -0.054 + 0.027644C - 0.000062C^2 - 0.016872t + 0.003037t^2 + 0.001619Ct \quad (2)$$

Table 2. Devices and chromatographic conditions.

HPLC	Shimadzu LC 10AT Vp series pump, Shimadzu SIL 10AD Vp cooling automatic sampling (4 °C), Shimadzu RF 10 AXL fluorescence detector (FLD), Shimadzu CTO 10AV Vp, Shimadzu SCL 10A Vp and Class Vp 6.14 (Shimadzu Corporation, Kyoto, Japan)
Injection volume	50 µl
Flow	0.8 ml/min
Eluent	40 phosphate buffer solution (1.36 g/l KH_2PO_4 (with H_3PO_4 (25%) adjusted to pH 3)), 60 acetonitrile
FLD	excitation wavelength 280 nm; emission wavelength 310 nm
Column	ACE C_{18} 250 × 4.6 × 5 µm
Oven temperature	35 °C
Analysis time	10 min

Where C is the concentration (mg/kg) and t is time (min).

The formula was validated by comparison with the results of an HPLC analysis of shrimp treated with 4-hexylresorcinol. The calculated values and the results of the analysis were similar (Table 3).

4. Conclusions

General research has shown that mathematical simulation formulae can be used in the fisheries and aquaculture industries. The use of mathematical simulations to calculate 4-hexylresorcinol residues in commercial shrimp production should ensure that these residues remain within legal limits. This method will allow the high cost of HPLC analyses of shrimp to be circumvented. Using this mathematical calculation to determine 4-hexylresorcinol residues will ensure that the high quality of the product and food safety standards are maintained, as the results of this simulation are similar to the results of HPLC analyses. Therefore, the use of this simulation should improve commercial practices and make them more consumer friendly.

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Table 3. The validation of the formula was accomplished by the comparison of HPLC analysis results of shrimps.

Repeat	Concentration/time	4-hexylresorcinol measured with HPLC detection (mg/kg)	4-hexylresorcinol calculated with developed formula (mg/kg)	4-hexylresorcinol variation (HPLC-mathematical formula)	
1	5 mg/kg	0.07	0.076846	-0.006846	*P*>0.05
2	1 min	0.08		0.003154	
1	5 mg/kg	0.16	0.114629	0.045371	*P*>0.05
2	5 min	0.15		0.035371	
1	5 mg/kg	0.26	0.298525	-0.038525	*P*>0.05
2	10 min	0.26		-0.038525	
1	50 mg/kg	1.31	1.241137	0.068863	*P*>0.05
2	1 min	1.00		-0.241137	
1	50 mg/kg	1.70	1.570344	0.129656	*P*>0.05
2	5 min	1.61		0.039656	
1	50 mg/kg	2.03	2.118519	-0.088519	*P*>0.05
2	10 min	2.21		0.091481	
1	100 mg/kg	2.31	2.242017	0.067983	*P*>0.05
2	1 min	2.35		0.107983	
1	100 mg/kg	2.69	2.895027	-0.205027	*P*>0.05
2	5 min	2.85		-0.045027	
1	100 mg/kg	3.92	3.847956	0.072044	*P*>0.05
2	10 min	3.85		0.002044	

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