

# The effect of a taste-enhancement process for cold-stored raw shell-stock oysters (*Crassostrea virginica*) on the spillage of human enteropathogens

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**Abstract** A taste-enhancement process for cold-stored, raw shell-stock *Crassostrea virginica* oysters (i.e., application of table salt to shells) when externally contaminated with human enteropathogens intensified spillage of these enteropathogens to oyster storage containers (77% compared to 27% for controls) but did not, however, cause contamination of edible oyster tissue.

## Introduction

*Cryptosporidium parvum*, *Giardia lamblia*, and human-virulent microsporidia (i.e., *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, and *E. hellem*) are human foodborne pathogens, which inflict considerable morbidity in healthy people (Weber and Bryan 1994; Franzen and Muller 1999).

Some of these enteropathogens, e.g., *Cryptosporidium* and microsporidia, can cause mortality in immunosuppressed individuals (Weber and Bryan 1994; Franzen and Muller 1999). *Cryptosporidium* and *Giardia* are transmitted via water (Franzen and Muller 1999; Thurston-Enriquez et al. 2002; Coupe et al. 2006) and are frequently reported from commercial oysters (Graczyk and Schwab 2000; Graczyk 2003). Numerous reports indicate involvement of water in the epidemiology of microsporidian spores (Sparfel et al. 1997; Dowd et al. 1998; Franzen and Muller 1999; Fournier et al. 2000; Thurston-Enriquez et al. 2002; Coupe et al. 2006), and their association with retail food (Calvo et al. 2004; Jedrzejewski et al. 2007). *Cryptosporidium* oocysts, *Giardia* cysts, and microsporidian spores are environmentally robust and therefore ubiquitous in coastal waters from which oysters are commercially harvested (Graczyk and Schwab 2000; Graczyk 2003; Graczyk et al. 2006; Słodkiewicz-Kowalska et al. 2006).

The popularity of seafood in the American diet continues to increase because seafood constitutes a healthy low-fat contribution of proteins into a balanced diet, and therefore raw oysters represent a highly popular and profitable seafood item (Graczyk and Schwab 2000; Graczyk 2003). Concerns have been raised regarding the health risks involved in consuming molluscan shellfish in raw form, partly due to the environmental presence of human pathogens in coastal waters (Graczyk and Schwab 2000; Graczyk 2003), and also because of potential natural microbial spoilage during storage (Andrews et al. 2000; Lorca et al. 2001; Cook et al. 2002; Gooch et al. 2002). The typical taste-enhancement for oysters destined for consumption on half-shells, involves making them “saltier”. One method is to sprinkle table salt on live oysters (shell-stock) before overnight refrigerated storage. This method is a simple, economic, and effective procedure that improves

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organoleptic quality of raw shell-stock oysters, enhances consumer enjoyment, and boosts oyster sales. The consequent spillage effects of human pathogens potentially present on oyster shell surfaces are, however, unknown. The purpose of the present study was to determine if the taste-enhancement procedure would cause the spillage of human enteropathogens from oyster shell surfaces to the edible oyster tissues.

## Materials and methods

Freshly harvested (Circle “C” Oysters Ranger Association, Ridge, MD, USA) commercial size (7.5–13.5 cm shell-length) Eastern oysters (*Crassostrea virginica*) were rinsed with sterile artificial sea water (ASW) of 12 ppt (Graczyk et al. 2006) and randomly separated in groups of 6 in 13 flat-bottomed plastic containers (30×50×5 cm). The containers were assigned into five groups of two containers and one group of three containers. Oysters in each group of two containers were individually sprayed with 6 ml of sterile ASW containing *C. parvum* oocysts, *G. lamblia* cysts, and *E. bienersi*, *E. intestinalis*, and *E. hellem* spores, respectively. Efforts were made not to spill the fluid on the bottom of the containers. The number of each enteropathogen was  $1.0 \times 10^4$ . The oysters in one container from each group were individually gently sprinkled with 3.0 g of table salt (World Finer Foods, Bloomfield, NJ, USA) with efforts made not to sprinkle salt on the container walls. Oysters from the group of three containers were individually sprayed with 3 ml of sterile ASW (first container), sprinkled each with 3.0 g of table salt, (second container), and left without any treatment in a third container. All containers were left overnight in dark at 4°C. Oysters were transferred to new containers, individually eluted with

10 ml of eluting fluid (Graczyk et al. 2007c), and the original containers were also eluted with 50 ml of the eluting fluid (Graczyk et al. 2007c). The oysters were shucked, and the resulting tissues of six oysters from each container were placed in a ziplock bag, left at 4°C for 2 h. The liquid fraction was decanted into 50 ml plastic tubes, and the flesh frozen at -20°C. The tubes were centrifuged (5,000×g; 10 min), the pellet collected and processed by sugar–phenol floatation (Ash and Orihel 1987). The eluting fluid was processed by the CAM-filter dissolution method (Graczyk et al. 1997). The resulting pellets from oyster and eluting fluid samples were processed by combined fluorescence in situ hybridization (FISH) and immunofluorescent antibody (IFA) method for *C. parvum* and *G. lamblia* (Graczyk et al. 2006; 2007a) and by the multiplexed FISH assay for microsporidian spores (Słodkiewicz-Kowalska et al. 2006; Graczyk et al. 2007b; Jedrzejewski et al. 2007).

## Results

Enteropathogens were not detected in any of the edible oyster tissue samples; however, they were identified in eluants obtained from oyster shells and containers (Table 1). Spillage from oyster shell to the container occurred irrespectively of the pathogen species or whether oysters were treated with salt (Table 1). Approximately 27% of pathogens delivered to the oyster shell end out on the container in the no salt option; this number significantly increased to 77% (chi-square test;  $F=5.4$ ,  $P<0.05$ ) when the salt was applied (Table 1). Salt on oysters caused significantly higher numbers of *C. parvum* oocysts and *G. lamblia* cysts to spill from the oyster shell to the container; however, there was no such effect for microsporidian spores (Table 1). Containers with salted oysters

**Table 1** The effect of a taste enhancement procedure for the Eastern oyster (*C. virginica*) on spillage of human enteropathogens from the shell surface

Pathogen	Number of pathogens eluted from			
	Oyster shells <sup>a</sup>		Plastic container <sup>b</sup>	
	A	B	A	B
<i>Cryptosporidium parvum</i>	3,000 <sup>c</sup>	800 <sup>c</sup>	750 <sup>d</sup>	2,800 <sup>d</sup>
<i>Giardia lamblia</i>	2,900 <sup>e</sup>	500 <sup>e</sup>	600 <sup>f</sup>	3,000 <sup>f</sup>
<i>Encephalitozoon bienersi</i>	3,700	3,500	930	900
<i>Encephalitozoon intestinalis</i>	2,900	3,100	820	800
<i>Encephalitozoon hellem</i>	2,100	3,000	790	890

Twelve oysters were individually sprayed with  $1.0 \times 10^4$  pathogens; 6 left as controls (A), and the other 6 individually sprinkled with 3.0 g of table salt (B), and all oysters kept overnight at 4°C.

<sup>a</sup> Cumulative number from six oysters.

<sup>b</sup> From containers in which oysters were kept.

<sup>c, d, e, f</sup> Statistically significant (chi-square test;  $F=5.6$ ,  $P<0.05$ ).

had approximately 5 ml of fluid on the bottom whereas containers holding unsalted oysters were merely moist. The mean volume of oyster liquid fraction was lower in salted oysters ( $28.2 \pm 4.5$  ml) than in oysters without salt ( $32.2 \pm 6.1$  ml).

## Discussion

In terms of public health, the present study demonstrated that human enteropathogens present on stored oyster shells did not contaminate edible tissues irrespective of whether the salt application, i.e., the taste-enhancement procedure, took place. In addition, the shucking procedure, similar to that used for the production of the half-shell oyster for consumers, did not result in contamination. This is despite the fact that the numbers of pathogens delivered to oyster shells (i.e., approximately  $8.3 \times 10^2$  per oyster) were much higher than potential natural contamination. Salt on oyster shells causes efflux of liquids from the shell crevices and from oyster tissue; thus preventing penetration of pathogens from the shell to the oyster inside. This physiological dewatering process makes the oyster flesh appreciably saltier to the enjoyment of consumers.

The present study showed, most importantly, that the commercially harvested oysters, destined for human consumption in a raw form, were free of these enteropathogens.

Proper storage of shell-stock oysters is essential for product safety (Andrews et al. 2000; Lorca et al. 2001; Cook et al. 2002; Gooch et al. 2002). Numerous studies have demonstrated that retail oysters contaminated externally can induce human infections due to natural aerobic spoilage during storage (Andrews et al. 2000; Lorca et al. 2001; Cook et al. 2002; Gooch et al. 2002). Post harvested oysters can contain more *Vibrio* than oysters at harvest time due to natural spoilage (Gooch et al. 2002). The present study demonstrated that seafood items could contaminate their environment during the storage (Graczyk et al. 2007c).

Application of salt on the oysters shell enhanced the spillage of *C. parvum* and *G. lamblia* from oyster shells, but not that of microsporidian spores. This is probably due to the fact that microsporidian spores have very small sizes (i.e., maximum 2.5  $\mu\text{m}$ ) comparable to the size of bacteria (Weber and Bryan 1994; Franzen and Muller 1999); this facilitates their penetration into porous oyster shell surfaces and also integration with shell microbial biofilm. *C. parvum* oocysts and *G. lamblia* cysts were also incorporated into the biofilm and shell surface; however, to a lesser extent than microsporidian spores, which are consequently more prone to spillage.

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