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Desirability of Oysters Treated by High Pressure Processing at Different Temperatures and Elevated Pressures

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ABSTRACT

Organoleptic changes in sterile triploid oysters (*Crassostrea virginica*) induced by high pressure processing (HPP) were investigated. Because pressure treatments evaluated were greater than current industry specifications, a volunteer panel composed of raw oyster consumers was chosen to evaluate desirability of HPP-treated oysters in a blinded test. Using a 1-7 hedonic scale, where 7 is "like very much" and 1 is "dislike very much", oysters were evaluated organoleptically for flavor, aroma, appearance, texture and general acceptability. The average acceptability score for untreated oysters was 4.64. Acceptability scores were 5.14, 5.13 and 5.28 at 300, 400 and 500 MPa for room temperature (22°C) pressure treated oysters, respectively. For oysters treated at 6°C, acceptability scores were 5.02, 5.53 and 5.38 for 400, 500 and 600 MPa treatments, respectively. Overall results indicate that HPP-treated oysters were preferred over non-HPP-treated oysters and that it is possible to perform HPP at ≥400 MPa which is required to inactivate shellfish-borne hepatitis A virus and human norovirus within shellfish meat, without a loss of oyster desirability. This study demonstrates that oysters treated by HPP under conditions that are known to inactivate human norovirus and hepatitis A virus would be accepted by consumers and therefore HPP can be a commercially viable intervention for virus contamination in raw shellfish.

Key words: High pressure processing, pre-pressurization temperature, taste panel, triploid oysters, virus inactivation

INTRODUCTION

High pressure processing (HPP), a food processing technique by which foods are placed under thousands of atmospheres of hydrostatic pressure, is an increasingly popular food processing technique (Knorr *et al*., 2011). HPP has a number of utilities for food processing which include inactivation of ripening enzymes such as for avocados permitting the production of guacamole with extended shelf life (Avomex; Saginaw, TX) and making unique foods based on starch and fish protein gels (Bauer and Knorr, 2005; Ohshima *et al*., 1993). But the principal interest in HPP has

been its potential ability to non-thermally inactivate foodborne pathogens while maintaining uncooked taste and character of foods. HPP is also used as a final lethality step for a number of ready-to-eat meats and deli products after packaging (Hayman *et al*., 2004). Because HPP is less effective against bacterial spores, the procedure is typically applied only to low pH foods and/or refrigerated foods. For shellfish, HPP is used commercially at pressures of approximately 300 MPa for less than 5 min at room temperature to facilitate shucking or separation of the meat from the shell (Martin and Hall, 2006) and for inactivation of *Vibrio vulnificus* (Berlin *et al*., 1999), a pernicious estuarine bacteria that can have serious medical consequences. Research has also shown the HPP can be effective against *Vibrio parahaemolyticus*, another estuarine pathogen (Kural *et al*., 2008).

Given that HPP is used commercially by the shellfish industry, there has been substantial research focus on its potential to inactivate pathogenic viruses within raw shellfish meat. Although, some uncommon viruses appear to be resistant (reviewed by (Kingsley, 2013)), HPP has shown excellent potential as an intervention for the hepatitis A virus (HAV) and human norovirus (HuNoV) which are currently the two principal virus threats to raw shellfish consumers.

Since human norovirus has not been successfully propagated to date (Herbst-Kralovetz *et al*., 2013), initial HPP inactivation research was performed with propagable HuNoV surrogate viruses, such as feline calicivirus and murine norovirus. Along with demonstrating the potential feasibility of HPP for norovirus within oysters and other foods, results showed that initial temperatures of approximately 5°C substantially increased inactivation as compared to initial pressurization temperatures at room temperature or higher (Chen *et al*., 2005; Gogal Jr. *et al*., 2011; Kingsley *et al*., 2002, 2007; Kingsley and Chen, 2008). Subsequently, a study assessing infection of human volunteers fed oysters injected with a total of 10^4 RT-PCR units of GI.1 human norovirus (8fIIb Norwalk) and then HPP treated demonstrated complete inactivation of human norovirus at 600 MPa and a 6°C pre-pressurization temperature and based on a reduced frequency of infection, demonstrated some HuNoV inactivation when HuNoV-injected oysters were treated at 400 MPa using a pre-pressurization temperature of 6°C (Leon *et al*., 2011). Investigation of GI.1 norovirus strain inactivation using a norovirus receptor binding (PGM-MB) assay suggested that 3-$\log_{10}$ of HuNoV were fatally damaged at 400 MPa when the initial pressurization temp was 5°C (Dancho *et al*., 2012). Adapting the PGM-MB assay to virus-contaminated oyster homogenate, (Li *et al*., 2013) have recently shown that a GII.4 norovirus, a strain thought responsible for approximately 80% of norovirus outbreaks (Siebenga *et al*., 2009), is in fact more sensitive to pressure than the GI.1 strain.

HPP can inactivate HAV within oysters and mussels but the treatments required are ≥400 MPa to inactivate ≥3 $\log_{10}$ of the virus (Calci *et al*., 2005; Kingsley *et al*., 2002; Terio *et al*., 2010), although, slightly less pressure (≥350 MPa) is sufficient within acidic foods, such as berry purees and chopped green onions (Kingsley *et al*., 2005). Curiously, inactivation of HAV by HPP is more efficient at room temperature (Kingsley and Chen, 2009; Kingsley *et al*., 2006).

Given that it is now clear that HPP can inactivate HuNoV and HAV within oyster meat, the purpose of this study was to determine how HPP-treated oysters perform organoleptically when treated under elevated pressures and altered temperatures required for virus inactivation. Because current industry specifications for HPP oysters are based partly on the idea that oysters processed at pressures above current standards would be unacceptable to the fresh raw oyster-eating public, we specifically chose a volunteer panel composed of raw oyster consumers to obtain their impressions in a blinded test. In this study, we report sensory evaluation of sterile triploid oysters

treated with current commercial HPP pressures and higher and compare results obtained with HPP-treated oysters pressurized at an initial temperature of 6 and 22°C.

MATERIALS AND METHODS

Oysters: Medium size (2.5-3 inches) sterile triploid oysters (*Crassostrea virginica*) were obtained from a local oyster farm (Atlantic Capes Inc., Cape May, NJ) during the month of August. Oysters were raised in full strength seawater. Sterile oysters were chosen for this study, because diploid reproductive oysters are typically emaciated at this time of year due to the metabolic demands of gamete production. Oysters were transported on ice to Virginia Tech for pressure treatment the following day, approximately 24 h after harvest.

HPP treatments: Whole-in-shell oysters were double bagged in 6.4×8.75 inch vacuum seal pouches (Thermo-Fisher Scientific, Newark, DE) approximately 24 h after harvest. Bags were heat-sealed using an Impulse Food Sealer (American International Electric Co., Whittier, CA) according to the manufacturer's instructions. Pressurization of oyster samples was performed for 5 min using a Quintus 35-L food press (QFP 35 L-600; Avure Technologies Inc., Kent, WA). Samples were pressurized at 300, 400 and 500 MPa for 5 min at pre-pressurization temperatures of 22°C and 400, 500 and 600 MPa for samples at pre-pressurization temperatures of 6°C. Oyster treatments at 6°C were placed on ice for at least 1 h prior to pressure treatment. Room temperature (22°C) treatments were equilibrated to room temperature for at least 1 h prior to treatment. Come-up times to reach final pressures ranged from approximately 75-135 sec for 300-600 MPa treatments. Pressure-release time was <3 sec. Non-pressurized oyster samples were sealed in vacuum pouches and stored on ice. The adiabatic temperature rise for HPP treatments starting at 6°C (400-600 MPa) ranged from 20-31°C and for HPP treatments starting at 22°C, adiabatic rise was 8-19°C (300-500 MPa) during pressurization. After processing, the samples were transported on ice to Delaware State University for organoleptic evaluation the following day.

Panelist: Volunteers participating ranged in age between 18-72 years and were recruited from the campus of Delaware State University and the local community. Volunteers recruited were believed to enjoy raw oysters and were instructed not to have any strong-tasting foods or liquids within 1 h of panel participation. Sixty-one panelists participated in the study. All volunteers reported that they were in good health and free of underlying health issues such as

Table 1: Demographic characteristics of sample

Characteristics	No.	Percentage
Gender	61	
Male	28	46
Female	33	54
Age (years)	60*	
18-30	16	26
31-50	21	34
51-72	23	38
Oyster consumption	61	
Frequently	2	3
Infrequently	21	34
Seldom	31	51
Almost Never	7	12

*: One panelist did not indicate age

immunosuppression, diabetes, blood or liver disorders, substance abuse and food allergies. Table 1 shows the demographic make-up of the panel. Prior to recruitment of participants, the study protocol was submitted to the DSU Institutional Review Board (IRB) and was rated "exempt."

Shucked on-the-half-shell oysters belonging to each of 7 treatment groups (non-treated; 300 MPa treated at 22°C, 400 MPa treated at 22°C, 500 MPa treated at 22°C, 300 MPa treated at 6°C, 400 MPa treated at 6°C and 500 MPa treated at 6°C) were individually fed to 61 volunteers in random order. Half-shell oysters were chilled and stored on ice prior to serving to volunteers. After each oyster, experimentally-blinded volunteers were asked to rate oysters on a 1-7 hedonic scale with 7 being "liked very much", 4 being "ambivalent" and 1 being "disliked very much." Hedonics included (1) Appearance, (2) Aroma, (3) Texture or mouthfeel, (4) Flavor and (5) General acceptability. After rating each oyster, volunteers were instructed to take a drink of water to cleanse their palates. All oysters were consumed 24-32 h after HPP treatment.

Statistical analysis: This study used the SPSS program to evaluate the data collected. Descriptive statistical analysis was performed to present the data. Analysis of data variance (ANOVA) was used to evaluate the effects of pressure and temperature (7 levels) on organoleptic scores. When appropriate, a Tukey's HSD (Honestly Significant Differences) *post-hoc* test was employed to check for differences amongst the means.

RESULTS AND DISCUSSION

Mean scores on 5 scales (appearance, aroma, texture, flavor and overall acceptability) for the seven oyster treatment groups were determined and are shown in Table 2. The average general acceptability score for untreated controls was 4.64, while room temperature (22°C) treated oysters scored 5.14, 5.13 and 5.28 for 300, 400 and 500 MPa treatments, respectively. For 6°C treatments, overall scores were 5.02, 5.53 and 5.38 for 400, 500 and 600 MPa treatments, respectively. Overall results indicate that HPP-treated oysters were actually preferred to non-treated control oysters and scored higher for all evaluation categories than the corresponding untreated oysters. Statistically significant preference ($p<0.05$) was observed between untreated controls and all pressure treated samples for "appearance" and a statistically significant preference ($p<0.05$) was observed for both the 6 and 22°C, 500 MPa treated oysters and the untreated control group for "texture". The acceptability rating for pressure-treated oysters was close to statistical significance ($p = 0.06$) as compared to untreated oysters.

The rationale for choosing individual treatment groups was as follows: Untreated oysters were evaluated to serve as a general baseline against which to evaluate organoleptic changes, the 300 MPa, 22°C treatment group was performed to mimic commercial HPP-treated oysters, 400 and 500 MPa, 22°C treatments were performed to characterize changes due to increased pressure at room temperature which might be utilized as a potential intervention for HAV; 400, 500 and 600 MPa, 6°C treatments were chosen because 400 MPa, 6°C is the pressure shown to substantially affect GI.1 and GII.4 HuNoV (Dancho *et al*., 2012; Li *et al*., 2013) and 600 MPa, 6°C was shown to inactivate 4-log RT-PCR units of GI.1 HuNoV, with 500 MPa, 6°C representing an intermediate treatment. The 600 MPa treatments at 22°C were not performed in this study because previous observations indicated that oysters treated under those conditions took on an obvious blanched appearance.

Table 2: Mean values for hedonic scale ratings for each characteristic and each sample (N = 56-61, depending on sample)*

Parameters	Control	300 MPa 22°C	400 MPa 22°C	500 MPa 22°C	400 MPa 6°C	500 MPa 6°C	600 MPa 6°C	F value (Sig. p-value)
Appearance	4.11±1.6^{a}	5.46±1.5^{b}	5.39±1.4^{b}	5.20±1.6^{b}	5.39±1.5^{b}	5.45±1.4^{b}	5.22±1.7^{b}	5.78 (0.000)
Texture	4.54±1.9^{a}	5.23±1.7^{ab}	5.36±1.8^{ab}	5.55±1.6^{b}	5.20±1.6^{ab}	5.47±1.5^{b}	5.43±1.7^{ab}	2.46 (0.024)
Flavor	4.64±1.7	5.04±1.8	5.05±1.7	5.13±1.7	4.86±1.6	5.35±1.6	5.27±1.6	1.24 (0.287)
Aroma	4.90±1.4	5.27±1.3	5.04±1.2	5.30±1.3	5.27±1.4	5.33±1.3	5.33±1.4	0.94 (0.469)
Acceptability	4.64±1.6	5.14±1.6	5.13±1.6	5.28±1.6	5.02±1.5	5.53±1.4	5.38±1.6	2.05 (0.058)

Rating scale: 7-Like very much, 1-Dislike very much, *Missing values for some samples ± values represent SD, Different letters indicate statistically different scores among treatment groups, statistical significance ($p<0.05$) was not observed for acceptability, aroma or flavor

For this study, several trends are observed. First, high pressure-treated oysters were preferred to non-treated controls. Manually-shucked oysters scored especially poorly in appearance. To some degree, this may have been influenced by the novice skill level of the person shucking the oysters, since some damage to the oysters' appearance by the shucking knife was noted. The degree to which this damage contributed to volunteer perceptions of aroma, texture, flavor, or general acceptability is unknown. Second, overall hedonic scores were similar for 6°C-treated oysters and for 22°C treated oysters. It was anticipated that oysters treated at 6°C would have higher overall scores than 22°C treated oysters based on the supposition that high pressure treatment at cooler temps would do less damage to oyster meat. Curiously, this was not the case. Third, it was anticipated that higher pressure treatments would score poorly as compared to lower pressure treatments and untreated control oysters. This was also not the case, as the 22°C oysters treated at 400 MPa and 500 MPa had slightly higher acceptability ratings than the 300 MPa treatment. For the 6°C HPP oysters, the overall acceptability rating did not decline for the higher pressure treatments of 500 and 600 MPa but for appearance, aroma and flavor, the 500 and 600 MPa treated oysters actually had higher scores, indicating greater acceptability compared to the 400 MPa treatment group.

The key question that this study hopes to address is can the pressure levels of oyster HPP treatments be raised to a level that will both inactivate HAV and/or HuNoV and yet still be commercially viable? Personal conversations with those working at commercial HPP operations have indicated that pressure treatments above 300 MPa have undesirable effects on raw oyster quality for both *Crassostrea viriginica* and *Crassostrea gigas* (Motavatit Seafood, Homa, LA and Nisbet Oyster Co, Bay Center, WA). This opinion also seems to be the general consensus of the shellfish industries. Curiously, it was reported in a landmark paper by Lopez-Caballero *et al.* (2000) that 400 MPa-treated *Crassostrea gigas* oysters were well-liked by Spanish consumers. We noted that Lopez-Caballero *et al.* (2000) performed pressure treatments at 7°C, unlike current industry practice which to our knowledge performs pressurization at ambient temperatures. We also noted that subsequent academic high pressure oyster studies such as He *et al.* (2002) do not cite a pre-pressurization temperature suggesting ambient room temperature was used, or such as Cruz-Romero studies that report initial pressurization at 20°C (Cruz-Romero *et al.*, 2004, 2007). Thus, to our knowledge, this is the first study performed at cooler initial pressurization temperatures to examine organoleptic changes to raw oysters above 400 MPa. We note that 500 MPa, 6°C received the highest overall acceptability rating of all oyster groups tested, suggesting that if industry finds a 300 MPa-treated oyster that was initially pressurized at room temperature commercially acceptable, it should also find a 500 MPa treated oyster that was

initially treated at refrigeration temperature acceptable as well. Also of key importance is that 400 MPa 6°C oysters had similar scores for acceptability, appearance, texture and flavor, compared to the commercial 300 MPa, 22°C oysters.

Given that HPP-treated oysters were clearly preferred in this study, there are a number of caveats that must be noted when discussing these results as compared to commercial HPP treatment of oysters. (1) In this study, whole-in-shell oysters were double-sealed in vacuum bags to keep the 35 L machine clean and prevent any oyster debris from contaminating the machine. Commercial pressure treatment is normally performed using whole-in-shell oysters held closed by a heat shrink wrap or a heat-sealed band. Although, shrink wrap does hold the shell closed, commercial heat sealing would not necessarily prevent contact between the oyster meat and fresh water used to create hydrostatic pressure within the pressure vessel. Thus the degree to which sealing in vacuum bags influenced these results is unknown. (2) These oysters were genetically triploid (n = 3) which are produced from diploid (n = 2) and tetraploid (n = 4) cross-matings. To our knowledge, this is the first sensory test performed on triploid oysters treated by HPP. Although, this is not believed to be an important distinction, it cannot be said for certain that diploid oysters would perform in an analogous fashion. (3) In this study, summer oysters were used. Traditionally in the mid-Atlantic region of the US, oysters are harvested in cooler months of the fall, winter and spring. It is conceivable that aquatic microflora and plankton are different in the warmer summer months, conceivably resulting in different tasting oysters. Also it is quite probable that the fatty acid composition of oyster meat would differ as a function of the water temperature in which the oysters were grown (Lira *et al*., 2013). (4) These were "high salt" oysters grown at the very southern end of the NJ peninsula, essentially in full strength seawater. Oysters readily grow in salinities exceeding 8 ppt to full strength seawater (~35 ppt) and their salt content mimics the ionic strength of the waters in which they are grown. It is known that higher ionic strength can influence the effectiveness of HPP (Kingsley and Chen, 2008, 2009). Whether this result is a function of the high salt profile of these oysters has not been determined. (5) Although, volunteers were recruited on the basis of generally liking to eat raw oysters, it cannot be said that all volunteers were oyster *Afficionados*. Whether more sophisticated oyster eating palates would score HPP-treated oysters in a similar manner remains to be determined.

Other issues that could influence the commercial suitability of HPP treated oysters are shelf life and drip loss, two factors which were not evaluated in this study. HPP treated oysters take up liquid water as a result of pressure effects on lipid cellular membranes resulting in swelling of oyster tissue. It is said that this results in HPP oysters being plumper and juicier. However, it is known that this liquid subsequently is lost after a few days. HPP is known to extend the refrigerated shelf life of oysters and clams and alter bacterial flora (Buyukcan *et al*., 2009; He *et al*., 2002; Linton *et al*., 2003) but how HPP treated oysters at refrigerated initial pressurization temperatures will perform as a function of time post-treatment has not been determined.

In summary, this study demonstrates that HPP performed at pressures above pressure levels currently used by industry can result in desirable oysters. These findings suggest excellent prospects for HPP as an intervention for HAV and especially for HuNoV which is more sensitive to HPP at cool temperatures.

CONCLUSION

In this study, the feasibility of applying increased pressure to HPP-treated oysters in terms of organoleptic changes in taste and texture is examined. Currently commercial HPP, a nonthermal technology, is performed to separate bivalve shellfish meat from its shell and as an intervention

for pathogenic *Vibrio* bacteria. However, previous research has shown that pressures >400 MPa can inactivate human norovirus and hepatitis A virus within shellfish meat. Here, it is determined that additional pressure does not substantially affect the taste and texture of oysters. Overall, this study demonstrates that higher pressures can be used as a food-borne virus intervention for HuNoV and HAV, without impacting consumer acceptance or commercial viability of raw HPP treated oysters. Also, it is determined that HPP performed on chilled oysters (6°C) resulted in acceptable oysters after 600 MPa treatments.

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