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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The impact of high-pressure processing on the growth of *Photobacterium phosphoreum* and biogenic amine formation in marinated herring

Yüksek basınç işleminin ringa marinatında *Photobacterium phosphoreum* gelişimi ve biyojen amin üretimi üzerine etkisi

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Abstract: The effects of high-pressure processing (HPP) on *Photobacterium phosphoreum* growth and biogenic amine formation were evaluated in marinated herring (prepared with 2% acetic acid+8% NaCl; or 4% acetic acid+8% NaCl solutions). Marinated fish fillets were inoculated with *P. phosphoreum*, vaccum packaged and treated with HPP in different pressure levels (100, 300, and 500 MPa) and pressure holding times (5 and 10 min). Control was left as untreated for both marination group. All batches were stored at 4±1°C up to 3 months. The results showed that combined effect of HPP and 4% acetic acid had much more inhibitory effect on the growth of *P. phosphoreum*, especially pressure levels 300 and 500 MPa. During the storage period, H_2S-producing bacteria growth was not observed in the groups subjected to 500 MPa pressure. Total psychrophilic bacteria did not grow in 500 MPa pressure treated group and 300 MPa 10 min pressure treated group prepared with 2% acetic acid during the storage period. Histamine was detected insignificant levels in the fillets marinated with 4% acetic acid and treated with HPP. Except for the control group tyramine formation was not found in the samples prepared with 4% acetic acid. Similarly, putrescine was not found in the samples prepared with 2% acetic acid and subjected to HPP treatment at the beginning of the storage. Cadaverine levels were found insignificant amount and 300 and 500 MPa pressure treatments suppressed the formation in 4% acetic acid treated groups compared with 2% acetic acid treated groups. The results of this study revealed that HPP in combination with 4% acetic acid had inhibitory effect on *P. phosphoreum* growth and suppressed the formation of histamine, tyramine, putrescine and cadaverine.

***Keywords*:** High pressure treatment, Photobacterium phosphoreum, herring, histamine, biogenic amine

Öz: Yüksek hidrostatik basınç işleminin (HPP) ringa marinatında (%2 asetik asit+%8 NaCl ve %4 asetik asit+%8 NaCl solüsyonları ile hazırlanan) *Photobacterium phosphoreum* gelişimi ve biyojen amin oluşumu üzerine etkileri değerlendirilmiştir. *P. phosphoreum* ile inoküle edilmiş marine edilmiş balık filetolarına, farklı sürelerde (5 ve 10 dk) ve farklı düzeylerde (100, 300 ve 500 MPa) basınç uygulanmıştır. Her iki marinasyon grubunda da kontrol basınç uygulanmadan bırakılmıştır. Tüm örnekler 4±1°C'de 3 ay depolanmışlardır. Sonuçlar, özellikle 300 ve 500 MPa basınç düzeyleri olmak üzere HPP ve %4 asetik asitin kombine etkisinin *P. phosphoreum* gelişimi üzerine daha fazla inhibitor etkisi olduğunu göstermiştir. Depolama boyunca 500 MPa basınç uygulanan gruplarda H_2S üreten bakteri gelişiminin olmadığını gözlenmiştir. %2 asetik asit ile hazırlanarak 300 MPa 10dk ve 500 MPa basınç uygulanan gruplarda toplam psikrofilik bakterilere depolama süresince gelişememiştir. %4 asetik asit ile hazırlanan ve HPP uygulanan gruplarda histamine düzeyi önemsiz seviyelerde bulunmuştur. %4 asetik asitle marine edilen gruplarda kontrol grubu dışında tiramin oluşumu gözlenmemiştir. Benzer şekilde %2 asetik asitle hazırlanan ve HPP uygulanan gruplarda da depolama başlangıcında putresin bulunmamıştır. Kadaverin miktarı önemsiz düzeylerde bulunmuş ve %2 asetik asit uygulanan gruplara kıyasla %4 asetik uygulanan gruplarda 300, 500 MPa basınç uygulaması kadaverin oluşumunu baskılamıştır. Bu çalışmanın sonuçları, HPP uygulaması ile %4 asetik asitin kombine bir şekilde kullanımının *P. phosphoreum* gelişimi ile histamin, tiramin, putresin ve kadaverin oluşumunu baskıladığını göstermektedir.

Anahtar kelimeler Yüksek basınç uygulaması, *Photobacterium phosphoreum*, ringa, histamine, biyojen amin

INTRODUCTION

Biogenic amines (BAs) such as histamine (HIM), cadaverine (CAD), putrescine (PUT), tyramine (TYM), spermidine (SPD) and spermine (SPM) are low-molecular-weight nitrogenous compounds. BAs are formed by means of decarboxylation of corresponding free amino acids by microorganisms which possess decarboxylase activity. Many bacteria species including enteric bacteria such as *Proteus vulgaris, Enterobacter aerogenes, Enterobacter cloacae, Serratia fonticola, Serratia liquefaciens* and *Citrobacter freundii* (Kim et al., 2003; Tsai et al., 2005) are responsible for BAs formation in seafood. In addition to them, *Morganella morganii, Klebsiella pneumoniae, Hafnia alvei* and *Photobacterium phosphoreum* have strong decarboxylase activity.

P. phosphoreum is a psychrotrophic and halophilic histamine producing bacteria which has high CO_2 resistance (Dalgaard, 2000). BAs formation and spoilage reactions in seafood can be prevented by conventional preservation techniques. However, these techniques such as chilling of seafood to 0-5°C are not sufficient alone to inhibit these

reactions. Therefore, additional preservation methods are required. High pressure processing (HPP) is minimal processing food preservation technologies that depending on the pressure, pressure holding time/temperature and product characteristics allows microbial inactivation at low temperatures with fewer changes in texture, colour and flavour of the product as than the conventional technologies (Ucak et al., 2018). HPP has been employed as a gentle pasteurization technique with generating high quality and microbiologically safe foods. The inactivation mechanism of microorganisms under HPP is based on destruction of membranes and cell walls, denaturation of proteins and enzymes in the cell membrane.

Despite its nutritional value such as high biological value proteins and lipids, fish is highly perishable due to high water activity, high level of unsaturated fatty acids and neutral pH (Lougovois and Kyrana 2005). The safety consumption of seafood is an important issue which can not be ignored by consumers and there has been increasing interest to extend the shelf-life and improving the microbiological quality.

Previous studies have shown that HPP can inhibit the microbial growth (Reyes et al., 2015; Gudbjornsdottir et al., 2010; Mengden et al., 2015; Kural and Chen, 2008a; Kural et al., 2008b; Kim et al., 2013) and can suppress the BAs formation (Matejkova et al., 2013; Krizek et al., 2014) in fish and fish products. Nevertheless, there are very limited reports on the effects of HPP on inhibition of *P. phosphoreum* and formation of BAs in fish product. Thus, this study was performed to determine the inhibitory effects of HPP on microbial growth and BAs formation in marinated herring storage at 4±1°C for 3 months.

MATERIALS AND METHODS

Bacterial strain

Photobacterium phosphoreum (DSM, 15556) were cultured in histidine broth (TSB supplemented with 0.5% L-histidine and 2.5% NaCl) at 20°C for 2-3 days. Early stationary phase cells were used and 10^6 CFU/mL bacteria cultures were prepared for the inoculation.

Preparation of fish marinade

Herring (*Clupea harengus*) fillets were purchased from fish market in Germany (Quakenbrück) and transported in ice boxes to the laboratory of German Institute of Food Technologies. Then fillets were put into polyethylene bags and stored at -20°C until using. For the marination two different solutions were prepared (2% acetic acid (v/v)+8% NaCl (w/v) and 4% acetic acid (v/v)+8% NaCl (w/v)) in the glass jars. The skins of thawed fish fillets were removed aseptically and rinsed with distilled water. Fish were placed into glass jars as 1:1.5 (w/v) fish-to-solution ratio. The ripening process was performed 4°C for 3 days. Marinated fish were removed from the solutions and drained for 30 min on a sterile bench.

Bacteria inoculation and HPP treatment

Marinated fillets were dipped into the *P. phosphoreum* culture solution for 5 min. The fish and bacteria solution ratio were 100g/mL. The inoculated fillets were vacuum packaged and kept at 2-4°C to prevent the temperature effects until the HPP treatment. The vacuum-packed marinated fish were treated with a high-pressure test system (WAVE 6000/55HT; NC Hyperbaric, Burgos, Spain) possessing a 55-L chamber and a maximum pressure level of 600 MPa. The pressure-transmitting medium was cold water (10°C) to maintain temperature conditions at room temperature during HPP treatment. For every 100 MPa increase in the pressure, the adiabatic heating of pressure transmitting fluid was 3-4°C. The compression and decompression times were not included in the treatment time. 100, 300 and 500 MPa pressure levels were applied for 5 and 10-min. Control was left as untreated for each marination group. All samples were stored at 4°C for 3 months and periodically evaluated.

Microbiological analysis

The microbial analyses were performed after HPP treatment and 15, 30, 45, 60, 75 and 90th days of the storage. 10g of fish were in a lab blender containing 90 ml pre-chilled sterile peptone physiological saline solution (0.1% peptone (w/v) + 0.85% NaCl (w/v)) for 60s. Further decimal serial dilutions were prepared from this homogenate in the same chilled sterile diluent. *P. phosphoreum* counts were enumerated by spreading of 0.1mL of the sample homogenate onto Long and Hammer agar. Then plates were incubated for 5 days at 15°C. H_2S-producing bacteria counts were determined by spreading of 0.1 mL of the sample homogenate onto Iron agar Lyngby (IA, Atlas 1997). Incubation period was at 15°C for 7 days. Black colonies were counted for enumeration. Total pscyhrophilic bacteria enumeration was conducted in Plate Count Agar (PCA) and plates were incubated at 7°C for 10 days (ICMSF, 1982).

Biogenic amine analysis

Ultra Performance Liquid Chromatography (UPLC-Thermo Scientific, Photodiode Array Detector) was used for the determination of histamine, tyramine, putrescine and cadaverine were conducted according to method of Eerola et al. (1993) with slight modifications. 15 mL 0.4 mol/L perchloric acid was added to 5.0 g of fish meat prior to homogenization for 1 min using an Ultra Turrax T25 (IKA-Labortechnik, Staufen, Germany). The homogenate was centrifuged (10 min, 2250 x g) and the supernatant passed through a 0.45 µm filter. After, 1 mL of sample extract was made alkaline by adding 200 µL 2 mol/L sodium hydroxide (NaOH) and buffered with 300 µL saturated sodium bicarbonate ($NaHCO_3$). Then, 1 mL of dansyl chloride ($C_{12}H_{12}ClNO_2S$) solution was added and the reaction mixture was incubated at 40°C for 45 min. Residual dansyl chloride was removed by adding 100 µL ammonia. After 30 min, mixture was adjusted to 5 mL with acetonitrile, filtered (0.45 µm, PTFE, MS Springer filter) and analyzed.

Statistical analysis

All measurements were carried out in triplicate and data were subjected to variance (ANOVA) analysis and Duncan's multiple range tests using the SPSS Version 18.0 statistical package (SPSS Inc., Chicago, IL, USA). A difference was regarded statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Effects of HPP on growth of *P. phosphoreum*

The inhibitory effects of HPP on the viable cell counts of *P. phosphoreum* in marinated herring are given in Figure 1-2. At the beginning the viable cell counts were found as 2.64, 2.56 and 1.82 log CFU/g in control, 100 MPa 5 min and 100 MPa 10 min pressure treated samples marinated with 2% acetic acid, respectively. *P. phosphoreum* growth was not observed in 300 MPa 10 min and 500 MPa 5, 10 min pressure treated group untill 60th day of the storage, while bacteria growth was detected at 45th day of the storage in 300 MPa 5 min pressure treated group. Significantly lowest ($p<0.05$) cell counts were determined in 500 MPa 5, 10 min pressure treated groups (4.08 and 2.54 log CFU/g) followed by 300 MPa 10 min pressure treated samples (5.48 log CFU/g) at the end of the storage period.

In herring fillets marinated with 4% acetic acid, *P. phosphoreum* could not grow at the beginning of the storage. However, on the 15th day bacteria growth was observed in the control and 100 MPa 5, 10 min pressure treated groups and at the end reached 6.48, 6.05 and 5.97 log CFU/g, respectively. Bacteria population did not exceed 7 log CFU/g, which considered as limit value for fish species during the storage period in these groups. Until at the end of the storage period, viable cell counts were not detected in 300 MPa and 500 MPa pressure treated groups. Ruiz-Capillas et al. (2007) reported that HPP treatment does not always inactivate microorganisms completely but may injure a proportion of the population, and the recovery of the injured cells depends on the subsequent conditions.

The findings of present study are consistent with the Kim et al. (2013), reported that *P. phosphoreum* growth was not observed in 300 and 400 MPa pressure treated mackerel muscle. Uçak et al. (2019) found that *Morganella psychrotolerans* growth was inhibited by 300 MPa and 500 MPa pressure treatment in marinated herring. The observation of present study pointed out that HPP and 4% acetic acid combination had more inhibitory effect on the growth of *P. phosphoreum*.

Effects of HPP on H_2S-producing bacteria

Total counts of H_2S-producing bacteria in marinated herring under HPP are represented in Figure 3-4. Initially, viable counts were 2.46 and 2.37 log CFU/g in control and 100 MPa 5 min pressure treated herring fillets marinated with 2% acetic acid, respectively. Bacteria growth was detected in 100 MPa 10 min, 300 MPa 5 min and 300 MPa 10 min pressure treated groups on the 15th, 45th and 60th day of the storage, respectively. Nevertheless, H_2S-producing bacteria growth was not recorded in 500 MPa pressure treated groups.

In the fillets marinated with 4% acetic acid, H_2S-producing bacteria growth was inhibited in 300 MPa 10 min and 500 MPa pressure treated groups. At the end of the storage period, highest value was observed in control (6.91 log CFU/g), while the lowest ($p<0.05$) value was found in fillets subjected to 300 MPa 5 min pressure level (5.25 log CFU/g). H_2S-producing bacteria are responsible for the main deterioration in fish and fish products stored at anaerobic conditions. Dalgaard (1993) reported that H_2S-producing bacteria growth is inhibited by low pH. Herland et al. (2008) determined the H_2S-producing bacteria in ice stored cod fillet after 9th day of the storage and bacteria cells reached 3.97 log CFU/g on the 15th day of the storage.

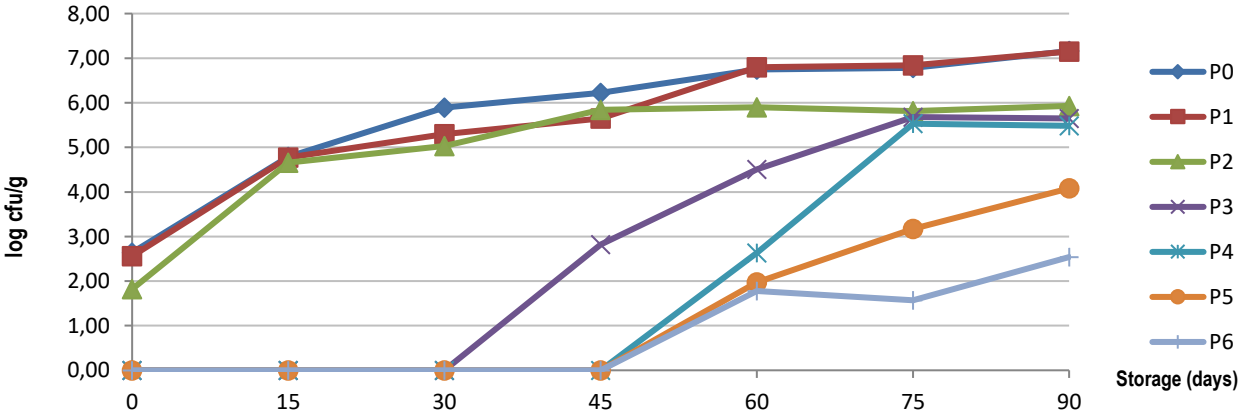

Figure. 1. Effect of HPP on the growth of *P. phosphoreum* in marinated herring prepared with 2% acetic acid and 8% NaCl. P0 (no HPP treatment), P1 (100 MPa 5 min), P2 (100 MPa 10 min), P3 (300 MPa 5 min), P4 (300 MPa 10 min), P5 (500 MPa 5 min), P6 (500 MPa 10 min)

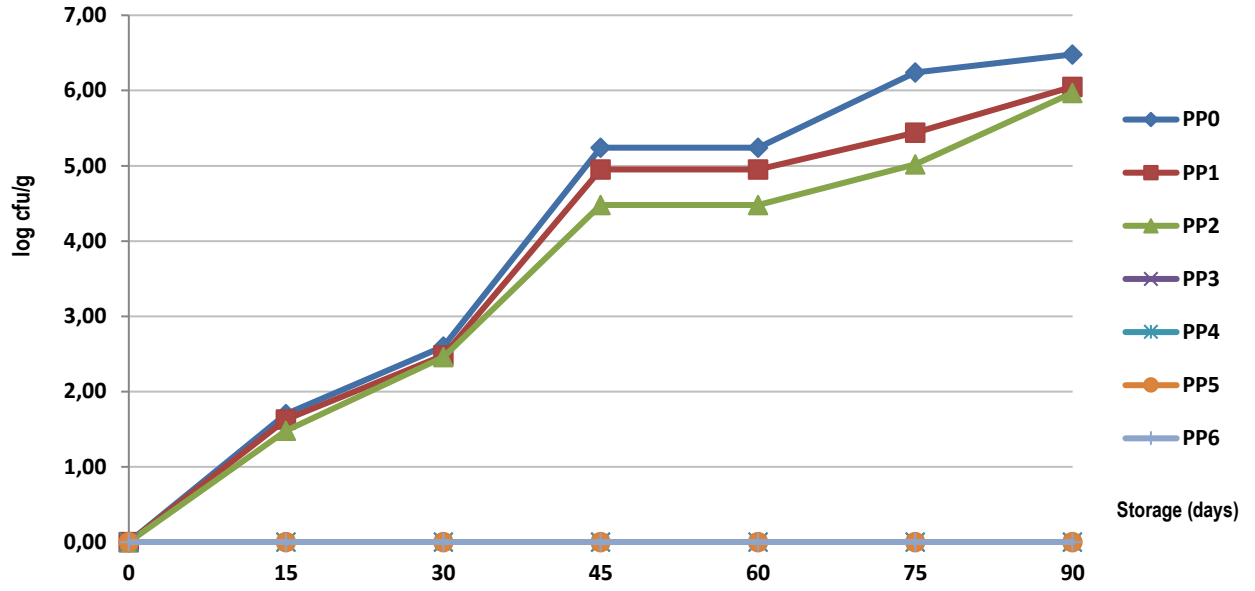

Figure 2. Effect of HPP on the growth of *P. phosphoreum* in marinated herring prepared with 4% acetic acid and 8% NaCl. PP0 (no HPP treatment), PP1 (100 MPa 5 min), PP2 (100 MPa 10 min), PP3 (300 MPa 5 min), PP4 (300 MPa 10 min), PP5 (500 MPa 5 min), PP6 (500 MPa 10 min)

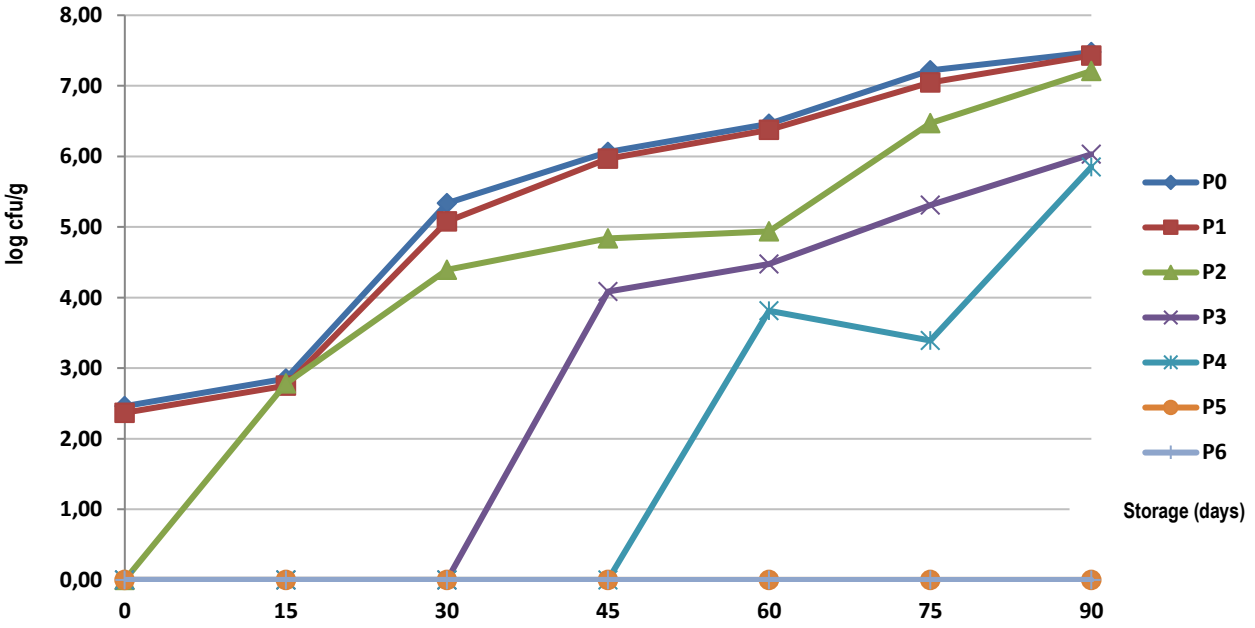

Figure 3. Effect of HPP on H_2S-producing bacteria growth in marinated herring prepared with 2% acetic acid and 8% NaCl. P0 (no HPP treatment), P1 (100 MPa 5 min), P2 (100 MPa 10 min), P3 (300 MPa 5 min), P4 (300 MPa 10 min), P5 (500 MPa 5 min), P6 (500 MPa 10 min)

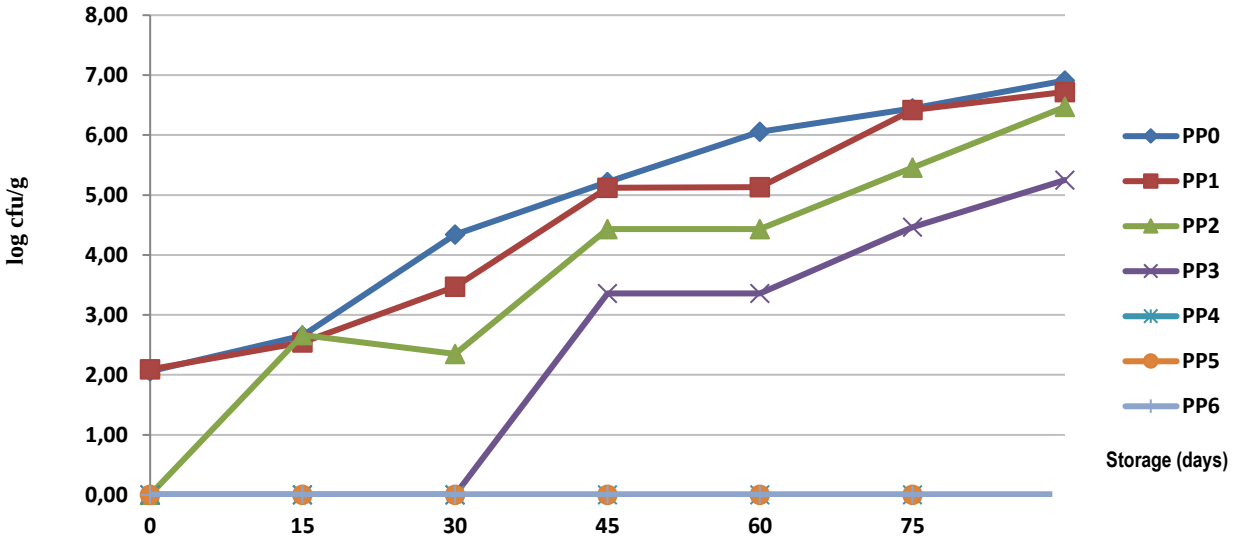

Figure 4. Effect of HPP on H_2S-producing bacteria growth in marinated herring prepared with 4% acetic acid and 8% NaCl. PP0 (no HPP treatment), PP1 (100 MPa 5 min), PP2 (100 MPa 10 min), PP3 (300 MPa 5 min), PP4 (300 MPa 10 min), PP5 (500 MPa 5 min), PP6 (500 MPa 10 min)

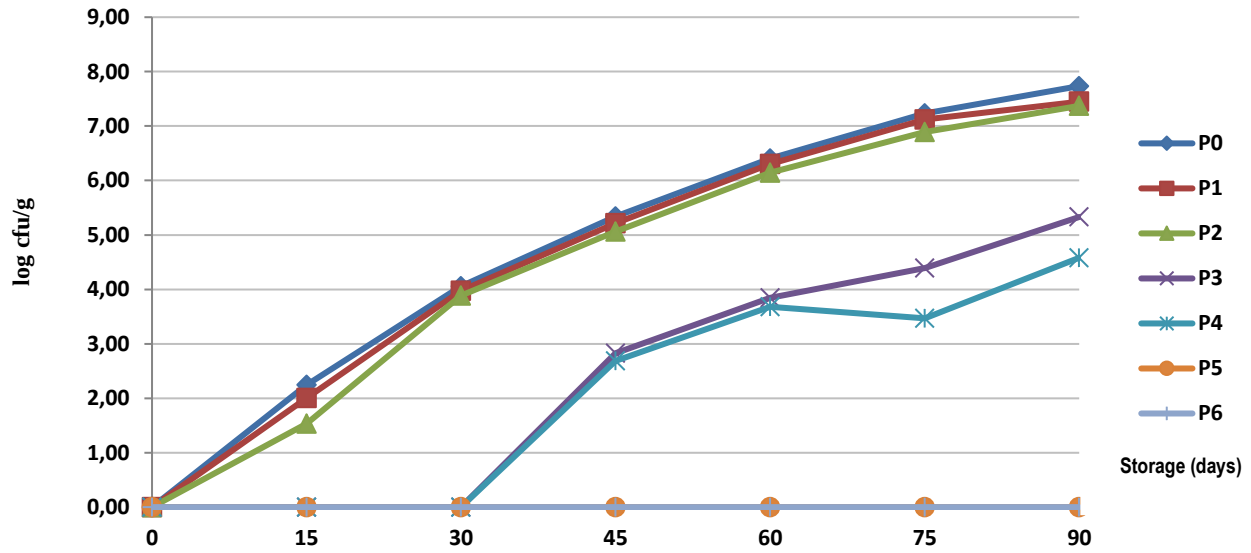

Figure 5. Effect of HPP on total psychrophilic bacteria growth in marinated herring prepared with 2% acetic acid and 8% NaCl. P0 (no HPP treatment), P1 (100 MPa 5 min), P2 (100 MPa 10 min), P3 (300 MPa 5 min), P4 (300 MPa 10 min), P5 (500 MPa 5 min), P6 (500 MPa 10 min)

Effects of HPP on total psychrophilic bacteria

Growth of total psychrophilic bacteria counts in marinated herring subjected to HPP are presented in Figure 5-6. In the aerobically stored fresh fish, gram-negative psychrotrophic bacteria are the main groups, causing the spoilage (Ibrahim Sallam, 2007). The initial total psychrophilic bacteria counts were found as 2.46 and 2.37 log CFU/g in control and 100 MPa 5 min pressure treated groups marinated with 2%, while bacteria growth was not observed in 100 MPa 10 min, 500 MPa 5 min and 300 MPa 10 min pressure applied groups until 15th, 45th and 60th days, respectively. Highest ($p<0.05$) viable counts were detected in control and 100 MPa pressure treated groups. In the groups subjected to 500 MPa HPP,

total psychrophilic bacteria growth inhibited during the storage period.

At the beginning of the storage, viable cells were not found in the groups prepared with 4% acetic acid and subjected to HPP. However, bacteria growth was observed in the control and 100 MPa pressure treated groups. During the storage period, total psychrophilic bacteria growth inhibited in the marinated herring treated with 300 and 500 MPa pressure level. Erkan et al. (2010) reported that total psychrotrophic bacteria count reached at 10^6 log CFU/g at 11 days in the red mullet fillets, while 330 MPa 5 min and 220 MPa 5 min HPP treated fillets reached this value at 17 and 15 days, respectively. Karim et al. (2011) found the initial total total psychrophilic bacteria count as 10^4 CFU/g in herring fillets and it was reported that pressure levels above 200 MPa had inhibitory effects during the storage. In another study Uçak et al. (2019) noticed that 500 MPa pressure treatment inhibited the total psychrophilic bacteria growth in marinated herring inoculated with *M. pychrotolerans*.

Effects of HPP on the biogenic amine formation

Histamine (HIM), tyramine (TYM), tryptamine (TRM), putrescine (PUT), and cadaverine (CAD) are the most important BAs in seafood associated with spoilage. Among them HIM and TYM are the most biologically active amines (Shalaby, 1996; Onal, 2007). Table 1-2 represented the effect of HPP treatment on the BAs formation in marinated herring. Initially, HIM level of control sample marinated with 2% acetic acid was 10.81 mg/kg and significantly ($p<0.05$) increased to 207.36 mg/kg at the end of the storage. In the HPP treated groups significantly highest ($p<0.05$) HIM level was observed in 100 MPa pressure treated fillets, while the lowest values were found in the groups subjected to 500 MPa HPP treatment. 4% acetic acid and HPP combination was more efficient in suppressing the HIM formation. HIM content exceeded the recommended level of 50 mg/kg by the Food Drug Administration (FDA, 2011) in control and 100 MPa 5 min pressure treated fillets marinated with 2% acetic acid after 30 days. Whereas, HIM was detected insignificant levels in herring fillets marinated with 4% acetic acid and treated with HPP.

At the beginning TYM levels were 2.27, 1.88 and 0.47 mg/kg in control, 100 MPa 5 min and 100 MPa 10 min pressure treated fillets and marinated with 2% acetic acid, respectively. TYM level exceeded the suggested acceptable limit for adults (100-800 mg/kg) by Ten Brink et al. (1990) in those groups at 30thday. TYM formation was not observed in 300 MPa and 500 MPa HPP treated fillets until 30th and 60th days, respectively. Significantly lowest ($p<0.05$) TYM values were detected in the groups treated with 500 MPa pressure level, while the highest values were observed in the control followed by 100 MPa and 300 MPa pressure treated samples. Except for the control group TYM formation was not found in the samples prepared with 4% acetic acid. This situation explains that all pressure levels succeded to suppress TYM formation.

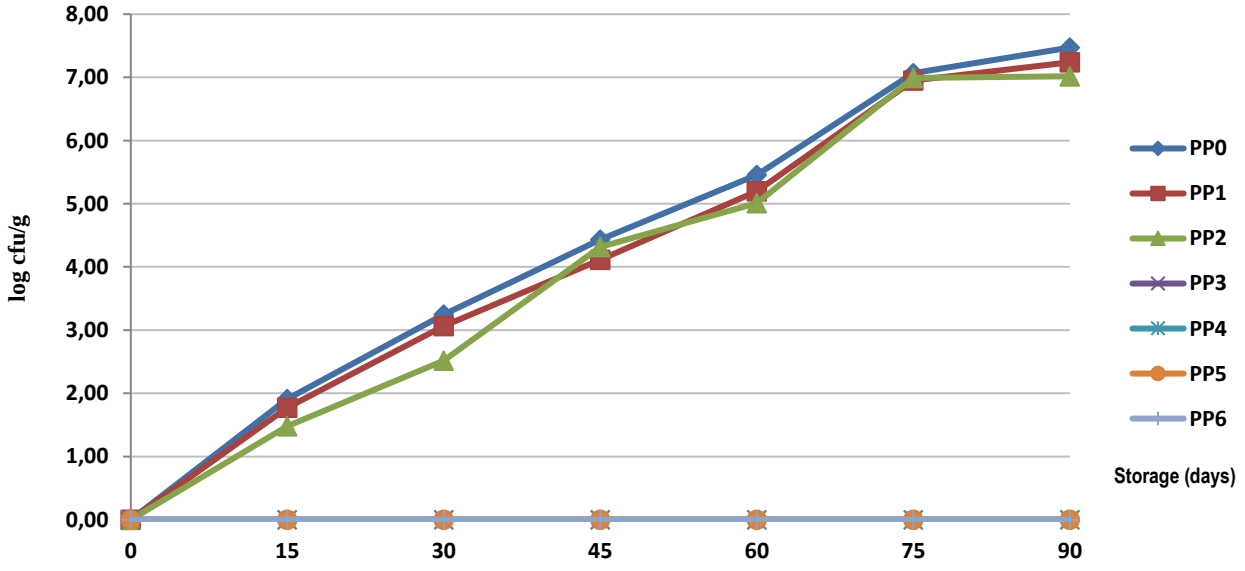

Figure 6. Effect of HPP on total psychrophilic bacteria growth in marinated herring prepared with 4% acetic acid and 8% NaCl. PP0 (no HPP treatment), PP1 (100 MPa 5 min), PP2 (100 MPa 10 min), PP3 (300 MPa 5 min), PP4 (300 MPa 10 min), PP5 (500 MPa 5 min), PP6 (500 MPa 10 min)

Table 1. Effect of HPP treatment on histamine and tyramine formation in marinated herring during storage (mg/100g)

Biogenic amines	Marination treatment	HPP treatment	Storage time (days)			
			0	30	60	90
Histamine	2% acetic acid	P0	10.81±0.02^{aD}	25.63±0.00^{aC}	116.41±0.07^{aB}	207.36±0.00^{aA}
		P1	8.54±0.04^{bC}	4.05±0.01^{bD}	110.99±0.05^{bB}	163.73±0.02^{bA}
		P2	7.71±0.03^{cC}	5.96±0.07^{cD}	9.51±0.14^{cB}	144.41±0.05^{cA}
		P3	4.63±0.00^{cC}	3.97±0.00^{dD}	7.19±0.10^{dB}	22.36±0.17^{bA}
		P4	6.43±0.01^{fA}	3.70±0.04^{eB}	3.68±0.09^{eC}	2.10±0.08^{eD}
		P5	4.73±0.06^{dA}	3.10±0.11^{fB}	1.90±0.27^{fC}	0.49±0.10^{fD}
		P6	4.63±0.13^{eA}	1.48±0.13^{gB}	0.94±0.01^{gC}	0.45±0.01^{gD}
	4% acetic acid	PP0	7.50±0.14^{aB}	4.74±0.04^{aD}	6.58±0.11^{aC}	8.36±0.03^{eA}
		PP1	4.71±0.05^{bC}	3.39±0.21^{bD}	5.87±0.05^{bB}	6.41±0.01^{eA}
		PP2	2.82±0.02^{cD}	2.89±0.06^{cC}	3.75±0.07^{cB}	4.82±0.16^{dA}
		PP3	2.36±0.01^{dA}	2.07±0.03^{dB}	ND	0.71±0.11^{dC}
		PP4	1.95±0.00^{eA}	1.58±0.15^{eB}	ND	0.71±0.09^{cC}
		PP5	1.10±0.06^{fB}	1.21±0.12^{fA}	ND	ND
		PP6	1.11±0.03^{gA}	0.62±0.09^{gB}	ND	ND
Tyramine	2% acetic acid	P0	0.47±0.05^{cD}	465.53±0.17^{bA}	462.65±0.12^{dB}	382.13±0.01^{eC}
		P1	2.27±0.07^{aD}	450.87±0.08^{cB}	508.98±0.37^{aA}	410.57±0.08^{cC}
		P2	1.88±0.27^{bD}	469.23±0.12^{aA}	437.72±0.01^{eB}	391.08±0.13^{dC}
		P3	ND	393.65±0.20^{dC}	541.81±0.08^{bA}	480.47±0.24^{bB}
		P4	ND	3.65±0.13^{eC}	535.63±0.03^{cA}	509.93±0.27^{aB}
		P5	ND	ND	1.49±0.23^{gB}	4.63±0.05^{fA}
		P6	ND	ND	3.20±0.32^{fA}	0.87±0.19^{gB}
	4% acetic acid	PP0	ND	4.48±0.04^{C}	23.36±0.16^{A}	20.46±0.11^{B}
		PP1	ND	ND	ND	ND
		PP2	ND	ND	ND	ND
		PP3	ND	ND	ND	ND
		PP4	ND	ND	ND	ND
		PP5	ND	ND	ND	ND
		PP6	ND	ND	ND	ND

ND: Not detected; Means indicated by di ferent lowercase letters in the same column differ significantly ($p<0.05$). Means indicated by different capital letters in the same row differ significantly ($p<0.05$). Each acetic acid group (2% and 4%) evaluated in itself.

Table 2. Effect of HPP on putrescine and cadaverine formation in marinated herring during storage (mg/100g)

Biogenic amines	Marination treatment	HPP treatment	Storage time (days)			
			0	30	60	90
Putrescine	2% acetic acid	P0	ND	4.78±0.17^{cC}	12.76±0.23^{bB}	13.31±0.01^{aA}
		P1	ND	3.88±0.22^{dC}	8.73±0.11^{cB}	9.61±0.08^{cA}
		P2	ND	6.31±0.34^{aC}	18.02±0.32^{aA}	11.79±0.18^{bB}
		P3	ND	5.65±0.28^{bA}	4.97±0.02d^{B}	2.48±0.29^{dC}
		P4	ND	ND	4.43±0.08^{eA}	1.89±0.36^{eB}
		P5	ND	ND	ND	ND
		P6	ND	ND	ND	ND
	4% acetic acid	PP0	ND	ND	ND	ND
		PP1	ND	ND	ND	ND
		PP2	ND	ND	ND	ND
		PP3	ND	ND	ND	ND
		PP4	ND	ND	ND	ND
		PP5	ND	ND	ND	ND
		PP6	ND	ND	ND	ND
Cadaverine	2% acetic acid	P0	13.40±0.15^{bB}	7.12±0.01^{bD}	10.38±0.17^{cC}	18.14±0.05^{aA}
		P1	13.05±0.33^{cB}	10.82±0.36^{aC}	14.81±0.25^{aA}	8.23±0.07^{bD}
		P2	13.45±0.08^{aA}	6.00±0.07^{fB}	2.19±0.28^{fC}	1.29±0.14^{cD}
		P3	11.37±0.17^{dA}	3.99±0.02^{eC}	10.98±0.31^{bB}	ND
		P4	10.91±0.02^{eA}	2.77±0.03^{gB}	1.62±0.07^{gC}	ND
		P5	9.15±0.06^{fA}	6.57±0.09^{dB}	5.45±0.09^{dC}	ND
		P6	9.00^{gA}	6.36±0.15^{cB}	2.09±0.04^{eC}	ND
	4% acetic acid	PP0	11.56±0.37^{aA}	8.70±0.05^{aB}	4.97±0.46^{aC}	3.54±0.02^{aD}
		PP1	7.41±0.26^{dA}	7.19±0.28^{dB}	4.52±0.15^{cC}	2.31±0.06^{fD}
		PP2	10.92±0.18^{bA}	8.46±0.16^{cB}	2.35±0.27^{fD}	3.13±0.01^{bC}
		PP3	6.35±0.06^{gA}	5.56±0.02^{fB}	2.31±0.02^{gD}	3.55±0.14^{aC}
		PP4	9.31±0.12^{cA}	8.49±0.09^{bB}	4.37±0.09^{dC}	2.52±0.22^{eD}
		PP5	6.74±0.03^{eA}	5.86±0.32^{eB}	4.91±0.17^{bC}	2.67±0.37^{cD}
		PP6	6.48±0.42^{fA}	4.79±0.252^{gB}	4.05±0.13^{eC}	2.64±0.12^{dD}

ND: Not detected; Means indicated by different lowercase letters in the same column differ significantly ($p<0.05$). Means indicated by different capital letters in the same row differ significantly ($p<0.05$). Each acetic acid group (2% and 4%) evaluated in itself.

The inhibitory effects of both 4% acid concentration and HPP treatment on the PUT formation were clearly visible, since there was no PUT formation in those groups (Table 2). Similarly, PUT was not detected in the samples prepared with 2% acetic acid and subjected to HPP treatment at the beginning of the storage, however, except for 500 MPa pressure treated groups PUT was observed after 0 day and reached the highest value in control.

CAD formation was found insignificant ($p>0.05$) levels in all groups during the storage period. The highest CAD level was recorded in both 4% and 2% acetic acid treated controls compared with the HPP treated marinated herring fillets, whereas the lowest values were determined in 300 and 500 MPa pressure treated groups. According to results, 100 MPa HPP treatment did not significantly affected the CAD formation in marinated herring fillets.

The results of the present study are consistent with other studies who reported that 500 MPa pressure treatment is more effective to suppress the PUT and TYM formation (Matejkova et al., 2013; Krizek et al., 2014). Ucak et al. (2019) reported significantly lower HIM formation in the marinated herring and treated with 300 and 500 MPa preesure compared with the control samples. According to another study HPP application significantly inhibited the HDC activity (Kim et al., 2013).

CONCLUSIONS

BAs can serve as indicators of fish spoilage since their presence in fresh fish is very low. *P. phosphoreum*, which is an important histamine producing bacteria, was inhibited in 300 and 500 MPa pressure treatment. Especially, the high acid concentration and HPP application combination was very efficient in inhibition of bacteria growth. The results of present study showed that the BAs content of marinated herring can noticeably reduced by the application of HPP. 4% acetic acid and HPP combination was more effective in suppressing the HIM, TYM, PUT and CAD formation.

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