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Extending shelf life of desalted cod by high pressure processing

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Abstract

Salted fish need to be rehydrated before eaten, but rehydrated fish have a relatively short shelf life. To increase the shelf life the products can e.g. be frozen, packed in modified atmosphere or processed by high pressure (HPP). Here, rehydrated cod was packed with different packaging regimes; in vacuum, with CO_2 emitter or in modified atmosphere [MAP], either alone or in combination with HPP. A shelf life study was performed, and headspace gas composition, drip loss, pH, colour, texture and microbial counts were assessed in the packaged and processed portions. The results showed that a shelf life of minimum 49 days can be obtained by treating the rehydrated cod by HPP or by combining HPP with modified atmosphere or in combination with a CO_2 emitter. The results of this study have shown that different packaging and processing methods can increase the shelf life of desalted cod.

Industrial relevance:

The growing trend and availability of "ready to cook" products are forcing the food industry to increase production of convenience products. Today desalted saltfish or clipfish need to be consumed immediately, stored chilled for a few days, stored frozen or being packed and/or processed to extend its shelf life. The use of high pressure processing represents a promising strategy to enhance the shelf-life of fish products. This study shows that HPP alone or packed with CO_2 can extend the shelf life of these rehydrated cod. Hence, these results can open up for new products.

1 Introduction

Salt-curing and drying of fish have been used as a traditional way of preserving fish since ancient times. Fully salted cod (saltfish) has a high salt content (~20%) and is low in water (~50%). The water content can be further reduced by drying (< 50%), and then dried salt-cured cod (clipfish) is obtained (Bjorkevoll, Olsen, & Skjerdal, 2003). Saltfish and clipfish can be stored for a long time even at abuse temperatures. Salted cod is split or fileted and then pickle salted or brine cured (Andres, Rodriguez-Barona, & Barat, 2005). To obtain clipfish, the salted fish is dried. Due to the very high salt content, a desalting or rehydration process, where the fish is soaked in water, must be performed before human consumption. Following the rehydration process substantial changes of the muscle are observed, and the product get the water content increased to 70–85% (w/w) and reduced its salt content to 2–3 % (w/w) (Lorentzen, Ytterstad, Olsen, & Skjerdal, 2010; Thorarinsdottir, Arason, Geirsdottir, Bogason, & Kristbergsson, 2002). Salt curing implies prevention of bacterial growth, and after a desalting process these products are favorable conditions for bacterial growth. Several studies (Barat et al., 2006; Bjorkevoll et al., 2003; Magnusson, Sveinsdottir, Lauzon, Thorkelsdottir, & Martinsdottir, 2006) have reported high bacterial counts (> 6 $\log_{10}$ cfu/g) in desalted products after 6 days of chilled storage (1–4°C), and with sensory rejection after 7–10 days (Bjorkevoll et al., 2003; Magnusson et al., 2006). The desalting of salted fish has traditionally been carried out in the household, but the trend with “ready to cook” products are increasing forcing the industry to increase production of convenience products that simplify everyday cooking. Today rehydrated saltfish or clipfish need to be consumed immediately, stored chilled for a few days, stored frozen or being packed and/or processed to extend its shelf life.

Bacterial growth in desalted fish can be inhibited by the use of modified atmosphere packaging (MAP), where the carbon dioxide (CO_2) packaging gas is used with or without oxygen or nitrogen (Aas, Skjerdal, Stoknes, & Bjorkevoll, 2010; Rotabakk, Sivertsvik, & Birkeland, 2009). The use of CO_2 emitters, that produce CO_2 gas in contact with water/liquid from the food, can also be utilized for quality preservation and shelf life extension (Hansen, Mørkøre, Rudi, Olsen, & Eie, 2007). HPP have previously been used in combination with CO_2 for inactivation of pathogens and extending the shelf life of food, though the variation in pressure levels used are high, varying from 48-600 MPa (del Olmo, Calzada, & Nunez, 2014; Garcia-Gonzalez et al., 2009; Lerasle et al., 2014; Liu et al., 2015; T. M. Rode, Hovda, & Rotabakk, 2015; Zhao et al., 2017).

High pressure processing (HPP) of fish and other seafoods have a great potential, related to shelf life and sensorial properties, and several studies confirm this. There have been several publications where HPP have been used on cod. We and others have shown that HPP can extend the shelf life of cod products (Arnaud, de Lamballerie, & Pottier, 2018; Montiel, De Alba, Bravo, Gaya, & Medina, 2012; Tone Mari Rode & Hovda, 2016), and the use of pressure is also shown to be advantageous in the desalting process (Salvador, Saraiva, Fidalgo, & Delgadillo, 2013). The use of HPP to extend the shelf life and eliminate pathogens in fish and desalted fish makes it advantageous since it preserves the natural and sensory properties of the products as reviewed by Oliveira et al. (Oliveira, Neto, Santos, Ferreira, & Rosenthal, 2017). High-pressure processing of food can be used on a variety of foods, both on existing products but also on creating new products.

The aim of this study was to investigate the effect of different packaging methods (vacuum, MAP and CO_2 emitter) alone or in combination with HPP for shelf life extension of

rehydrated clipfish and saltfish. We have done a shelf life study and included several analyses like colour, texture, drip loss, pH and microbiological analysis.

2 Materials and methods

2.1 Samples

Two types of fully salted Atlantic cod was used; (a) Clipfish – skinned and boned filet that were salted and dried. The loin fillets were portioned before rehydration; and (b) Saltfish – fish that was fully salted and then portioned, including skin and bones, to approx. 5 x 21 cm. The fish pieces weighed between 100–150 g and 231–412 g for clipfish and saltfish, respectively.

2.2 Rehydration

The rehydration was carried out in plastic containers with a lid stored in a cooling rom at 4 °C. Due to the difference in weight and dryness, different rehydration procedures were utilised. For clipfish (a) – The loin pieces were rehydrated (fish : water [w/w] = 1 : 6) for 48 h. After 6, 11 and 24 h, the water was changed and replaced with precooled water. For saltfish (b) – Lombos pieces (from the upper part of the fish) were rehydrated (fish : water [w/w] = 1 : 9) and the water was stirred once per day (after 24, 48 and 72 h). The total rehydration time was 96 h, and no water change was performed. At selected time points during the rehydration process (clipfish: 6, 11, 24 and 48 h; and saltfish: 24, 48, 72 and 96 h) the weight of eight samples were measured. The total weight of the desalted portions was also recorded after rehydration.

2.3 Packaging treatment

The rehydrated saltfish was after draining put in plastic bags; (1) with no sealing; (2) vacuum packed; (3) with a CO_2 emitter and vacuum packed; or (4) packed in modified atmosphere (MAP) with 60 % CO_2. In addition, samples were packed in (5) vacuum; (6) CO_2 emitter and vacuum; and (7) MAP and thereafter processed by high pressure. The CO_2 emitter (Vartdal Plastindustri AS, Norway) used in the experiments produced 245 ml of CO_2 by absorbing 55 ml of added water. All samples were packed (Webomatic vacuum chamber, Werner Bonk, Bochum, Germany) in heat-sealed 20 mm PA/70 mm PE bag (180 × 140 mm, Lietpak, Vilnius, Lithuania). The oxygen transmission rate for the bag was 0.9 $cm^3d^{-1}atm^{-1}$, measured at 296 K, 75% RH. Vacuum packed samples were packed in 99 % vacuum. Rehydrated clipfish was, after drainage, vacuum packed individually and stored on ice.

2.4 Pressure treatment

The rehydrated clipfish samples were treated at 400, 500 and 600 MPa for 5 min at ambient temperature, approx. 19–20 °C. Additionally, some samples were processed at 600 MPa for 2 and 10 min. The rehydrated saltfish samples being exposed to high pressure processing (HPP), sample # 5–7, were treated at 600 MPa for 5 min at 8–9 °C. The samples were pressurised in a high hydrostatic pressure machine QFP 2L-700 (Avure Technologies Inc., Columbus, USA). Come up time was approx. 65 and 100 s for 400 and 600 MPa, respectively, whereas the pressure release was immediate. The duration of treatment did not include the come-up time. The rest of the samples were non-pressurised control samples (0.1 MPa). Prior and after treatment, samples were kept on ice.

2.5 Storage

All samples, both pressurised and non-pressurised ones, were stored at 4 °C in the dark in a cooling cabinet. A temperature sensor was logging the temperature the whole storage period.

2.6 Analysis

The desalted clipfish and saltfish were analysed on day 0, both before and after HPP. In addition, clipfish HPP-samples were analysed at day 15 and 49, for drip loss, pH, colour, texture and microbiological analysis. Non-processed samples were analysed at day 4. For saltfish, microbial analysis and measuring head space gas, drip loss and pH was performed. Non-pressurised samples were analysed day 0, 5, 11 and 15, and the HPP-samples were analysed at day 0, 15, 30, 40 and 49.

2.6.1 Headspace Gas Analysis

The headspace gas composition (O_2 and CO_2, % concentration) was assessed (n = 3 for each treatment) by using an oxygen and carbon dioxide analyzer (Checkmate 9900 analyzer, PBI-Dansensor, Ringsted, Denmark). An aliquot (20 mL) of the headspace gas of the package was collected with a syringe after intrusion of the top film. Before intrusion of the syringe, a foam rubber septum (Nordic Supply, Skodje, Norway) was fastened to the top foil to avoid introduction of false atmosphere into the package. It was not possible to make measurements of samples packed with CO_2 emitter since they were vacuum packed.

2.6.2 Assessment of Salt, Drip Loss, and pH

The salt concentration in the fish was measured as total chloride content by titration with silver chloride (Mettler Toledo T7), according to ISO 5943 IDF 88:2006 with some deviations. Homogenized samples (1 g, n = 3) were diluted with deionized water (50 ml, heated to 55 °C), left for 1 h without subsequent blending, and titrated in an automated titration unit. Salt

concentration in the water (%) was measured in each desalting unit, after stirring, and before any water change using a manual salt analyzer, an analog refractometer (Kern Optics, Balingen, Germany). Duplicate samples of water were analysed. The drip loss during storage (%), the weight of exudates in the trays after storage in relation to the original weight of the portion, was calculated from triplicate samples. pH was measured in room tempered, homogenised samples using a pH-meter instrument PHM210 (Meterlab, Copenhagen, Denmark).

2.6.3 Colour

The surface colour (L*, a* and b* values, CIELAB) was assessed by a calibrated digital photo imaging colour-measuring system (DigiEye full system, VeriVide Ltd., Leicester, UK). Samples were placed in a standardized light-box with daylight (6400 K) and photographed with a calibrated digital camera (Nikon D80, 35 mm lens, Nikon Corp., Japan). Pictures were analysed with DigiPix software (VeriVide Ltd., Leicester, UK) and the various color parameters were quantified. L* describes brightness (L* = 100 = white, L* = 0 = black), a* describes the intensity of the color in red-green axis (a* <0 = green, a*> 0 = red), while b* describes the intensity of the yellow-blue axis (b* <0 = blue, b*> 0 = yellow).

2.6.4 Texture

The texture analysis was performed with a TA.XTplus equipped with a 50 kg weigh cell and the software Exponent ver: 6.1.16.0 (Stable Micro Systems, Surrey, UK). A flat cylinder probe with a diameter of 1/2 '' (12.7 mm), Type P/0.5, was used. The probe was pressed down to 80 % of the sample height at a rate of 1 mm/sec, and the force at 20 %, 40 %, 60 % and 80 % compression of the initial height (firmness) were assessed at two locations on each sample. After opening the samples, the fish was wrapped in plastic and tempered at room

temperature for 1 h prior to analysis. Mean values of the two assessments were used for statistical analysis of the data.

2.6.5 Microbiological Analyses

Samples of muscle (25 g) were diluted 1 : 10 in peptone water (BactoPeptone, Merck, Darmstadt, Germany) added 0.85 % NaCl, and homogenised for 2 min in a Stomacher 400 Laboratory Blender (Seward Medical, London, UK). Aerobic quantification of psychrotrophic and heat labile microorganisms (colony forming units [cfu] ml^{-1}) was performed by surface plating on Long & Hammer agar (L&H) and Tryptic Soy Agar added 2 % NaCl (TSA-NaCl) plates. The plates were incubated for 5–7 days at 15 °C. A mechanical spiral plater (EddyJet, IUL Instruments, Barcelona, Spain) was mainly used for this purpose. However, some manual plating was performed for low dilutions. Aerobic count was also performed on Iron Agar to determine hydrogen sulphide producing bacteria. A pour plate technique was used, adding Iron Agar with 0.8 % L-cysteine to 1 ml of sample. Plates were incubated for 3–4 days at 20 °C. Both the total number (aerobic count) and the number of black colonies were counted (hydrogen sulphide producing bacteria). The detection level on L&H/TSA and Iron Agar was 10^2 and 10 cfu g^{-1}, respectively. For plates with no colonies detected, the level was set to half of the detection limit.

2.7 Statistical analyses

Statistical analysis included analysis of variance (one way ANOVA), general linear modelling (GLM) and Tukey's HSD test ($p<0.05$). All data processing was carried out on Minitab v19 (Minitab Ltd., Coventry, UK). To meet the requirements of equal variance and normal distribution, all statistical analyses of microbial growth were done on log-transformed data. The data provided are average values of a minimum of 3 values where the variations are shown as standard deviations, unless otherwise stated.

Each experiment has been repeated twice, performing rehydration on different days. The following analysis has been conducted with a minimum of three technical replicates for each production if nothing else is stated.

3 Results and Discussion

3.1 Weight change after rehydration and salt content

The rehydration time was different for the clipfish and saltfish. This must be seen in context with the size and the thickness of the fish, and the differences in processing (salting, drying). For the dried and salted clipfish, the average weight gain for 16 random fish pieces (2 experiments), followed during the rehydration process (48 h), was an increase of 17.7 ± 1.1 %. Highest weight change was after 6 h, with an increase of 7.3 %. Furthermore, an average increase of about 2.5; 4.2; and 2.7 % was observed after 11, 24 and 48 h. The average weight gain for 16 random saltfish pieces (2 experiments) during 96 h rehydration was 23.7 ± 2.7 %. The highest weight change was observed after the first 24 h, with an average increase of 14 %. Furthermore, an average increase of 4.1; 2.4; and 1.7 % was observed for the intervals; 24−48 h, 48−72 h, 72−96 h, respectively. The observed weight gain for the saltfish is similar to results that we and others have reported earlier (Andres et al., 2005; Rotabakk et al., 2009). Three water changes were made for the clipfish, and the salt content of the water was 0.9 ± 0.2 after 6 h. Furthermore, the salt level was 0.5 ± 0.1 after 11, 24 and 48 h. Due to the frequent water changes, it is not surprising that the salt content of the water was relatively low. The salt level in the rehydrated clipfish showed that there were relatively large differences between the different pieces. The average level was 3.8 ± 1.2 %. No water change was performed when rehydrating the saltfish. The salt content of the water after 24 h was 1.4 ± 0.1 %. Thereafter the salt level was 2.0 ± 0.1, 2.1 ± 0.0 and 2.3 ± 0.1 % after 48,

72 and 96 h, respectively. Since there was no water exchange, the transport of salt out of the fish muscle was fast the first 24–48 h. After that it went relatively slowly, as the fish and water came closer to an equilibrium. The salt content in the rehydrated saltfish was 2.8 ± 0.4 %.

3.2 Headspace gas composition in the saltfish packages

Only selected saltfish samples were packed in MAP. The gas composition in these bags (day 0) were 61.8 ± 0.8 % and < 0.01 % for CO_2 and O_2, respectively. After 5 and 15 days of storage, a decrease to 36 and 32 % CO_2 was observed. Further storage showed more than halving in CO_2 levels. At day 30, 40 and 50, the CO_2 levels were 24.2, 23.2 and 19.6 %, respectively. During this period (> 30 days), the O_2 values increased to around 1.1 %. The head space composition is an average of all MAP samples, both pressure and non-pressure treated. The CO_2 concentrations decreased significantly with increasing storage time. This was probably caused by diffusion of CO_2 through the packaging material, and a high barrier pouch should have been used as the storage time exceed 21 days. We have earlier also observed increase in O_2 level during storage of desalted cod in MAP (Rotabakk et al., 2009). In the saltfish packages containing CO_2 emitter, the headspace was too small to measure any gas composition but consisted most likely of an atmosphere close to 100 % CO_2.

3.3 Drip loss and pH during storage

Clipfish. All desalted fish were drained before packaging, but the vacuum-packed samples got some exudate after 1-2 h in a cooling room. The clipfish samples that had been exposed to HPP showed a significantly (p=0.008) higher drip loss after processing (3.1 ± 1.3 %) compared with the non-treated ones (1.5 ± 0.5 %) at day 0, Figure 1. This shows that a relatively modest drip loss (< 2%) was directly linked to the high-pressure process. The fluid

release for the untreated control samples show that an additional fluid release was obtained after the run-off period. No significant ($p=0.730$) differences between the pressurized samples were detected at day 0. After storage for 15 days, a significant ($p<0.001$) difference in drip loss was observed between the processed samples. Processing at 600 MPa had a significant increased fluid release (5.6 ± 1.2 %) compared to samples processed at 400 and 500 MPa (3.3 ± 1.0 %). After 49 days of storage (only 600 MPa), the fluid release was 4.1 ± 0.9 %. In other studies, we and others have observed around 2–4% exudate in MAP desalted cod after storage, day 15 and 24 (Magnusson et al., 2006; Rotabakk et al., 2009), and processing (500 MPa) of fresh cod is reported to give drip loss of 4.9 and 7.1 % after 11 and 18 days of storage (Christensen, Hovda, & Rode, 2017). The control had a 0.7 and 4.2 % drip loss at the same storage days. This shows that the drip loss of desalted cod after 49 days storage is at the same level as observed for vacuum packed fresh cod and for MAP desalted cod stored for 15–24 days. It is reported by us and others that HPP increase drip loss, both after processing and during storage compared with untreated control (0.1 MPa), and this has been linked to denaturation of myosin (Christensen et al., 2017; Truong, Buckow, Stathopoulos, & Nguyen, 2015). The water holding capacity (WHC) was not measured for the desalted cod, but one can speculate that the WHC would be reduced due to HPP and after storage compared with an untreated control. Christensen et al. (2017) reported that WHC decreased significantly after storage (11 and 18 days) for HPP fresh cod, while no changes were observed for untreated control after storage. In a review by Oliveria et al. (2017) it is reported that reduced WHC can be due to compression of fibers and protein denaturation, and that it is pressure dependent. At day 0 there were no significant difference between pressurized and control cod.

Saltfish. The samples that had been exposed to pressure had a significantly ($p<0.001$) increased drip loss compared with the corresponding non-pressurised samples. For saltfish packed with emitter and MAP, the drip loss was generally > 5% higher in pressurized compared to non-pressurised samples, Figure 2. Packaging in vacuum and MAP gave statistically significant ($p<0.001$) decrease in drip loss compared to saltfish packed with CO_2-emitter, regardless of HPP treatment or not. At day 15, the drip loss in the non-HPP samples were twice as high in the emitter-samples compared to the MAP-samples.

In general, the saltfish samples showed relatively small differences in drip loss during the storage period. Except between day 0 and the rest of the storage trial for HPP-treated samples, no significant ($p>0.345$) changes in the drip loss were observed during the storage period. Sivertsvik (2007) showed that drip loss of fresh farmed cod (*Gadus morhua*) packaged in modified atmosphere increased with increasing CO_2 in the head space. Even though the CO_2 in packages with emitter was not possible to measure, it can be assumed that those packages had the highest amount of CO_2, which can explain the observed increased drip loss in the emitter samples. The use of oxygen with CO_2 can have a positive effect on drip loss (Rotabakk et al., 2009), so including O_2 in the MAP could have a positive effect in reducing the drip loss.

pH in the clipfish varied from 6.12–6.30 at day 0, Table 1. In the stored samples (>5 days), saltfish under vacuum conditions, both with/without HPP treatment, showed a significantly ($p<0.001$) higher pH compared with the other packaging conditions. No significant ($p>0.05$) difference was observed between MAP and emitter. In the stored samples, both emitter and MAP had a significantly ($p<0.001$) decreased pH compared to vacuum, regardless of HPP treatment or not. This is most likely caused by the acidic effect of dissolved CO_2 (Coryne, 1933). For the HPP samples, there was a general decrease in pH,

except for day 40, during the 49 days of storage. There was no significant (p=0.589) effect of HPP treatment. The samples packed in vacuum, with/without HPP, showed a significantly higher pH compared with other packaging methods.

3.4 Colour and texture after processing and during storage of clipfish

The colour and texture analysis were only performed for the clipfish samples, since they were relatively even pieces of the loin with no bones. At day 0 there was no significant difference between the non-pressurised control and the HPP samples. In a previous study on fresh cod, statistically significant differences ($p<0.05$) in whiteness was seen for the non-pressurised samples, the lightness (L*) value was 75.9, compared with the samples treated at 500 MPa, where the whiteness was increased to 91.6 (Christensen et al., 2017). In this study the non-pressurised desalted cod showed L* values of 74.9, while HPP at 500 MPa increased the whiteness to 78.1 (not shown). This indicates small differences in whiteness of untreated (control) fresh cod compared to desalted cod. When comparing these studies, exposure to 500 MPa showed that differences is whiteness was very prominent for the raw cod while non-significant changes was detected for the desalted cod compared with the non-pressurised samples. A suggestion can be that some of the pressure sensitive proteins have already been denatured due to the salting and drying process of the cod, and therefore a non-significant change in lightness was detected when pressurizing at 500 MPa compared with the control (0.1 MPa). The colour of the pressurised samples was not significantly ($p>0.376$) different comparing the different pressure treatments, 400–600 MPa, so the values were pooled for the different storage times, Table 2. The colour parameter, L*, a* and b*, all showed significantly different values with increasing storage time. The samples got less white, more red and more yellow compared with the day 0 samples. Christensen et al. (2017) also observed a tendency of decreasing L* values (less whiteness) for fresh cod

processed at 500 MPa during storage, but the storage was ended after 18 days. Comparing the results of the desalted cod with the change in colour observed for raw fresh cod, it is our opinion that the colour changes were relatively minor, even though they were significantly different. Arnaud et al. (2018) have reported that changes in lightness due to HPP can be acceptable and even preferred by consumers.

For all compression rates, HPP made the samples significantly ($p<0.047$) harder than the untreated samples (data not shown). When comparing the data for the HPP samples, both storage time and pressure came out as significant main effects. All texture data are shown in Figure 5. Significant changes ($p<0.018$) at 20 % shows that the surface got a softer texture during the storage period of 49 days. However, only 600 MPa were analysed at day 49. Storage of fish over time generally gives softer texture, either due to enzyme activity or bacterial growth, or both. Due to the low bacterial growth at day 49, the softer tissue that was observed was most likely due to increased enzymatic activity. The latter is confirmed by others reporting that the texture of cod-like fish species is largely influences by enzymes, and can also be changes by HPP (Matser, Stegeman, Kals, & Bartels, 2000). No significant difference was found between day 0 and 14 on any of the samples. The texture analysis showed that the samples pressurised at 500 MPa gave the hardest texture among the other pressure levels tested.

3.5 Microbiological quality

There is often a relation between increased rehydration time and increased number of bacteria in the rehydrated product. The clipfish samples showed low bacterial counts after the rehydration process. And the processing at 500 and 600 MPa gave bacterial counts around 2 log cfu/g, Figure 3. After 15 days of storage, the bacterial load had significantly ($p<0.001$) increased in the samples processed at 400 and 500 MPa. After 49 days of storage

only the samples treated by 600 MPa were analysed, and no significant ($p=0.493$) increase in bacterial levels throughout the whole storage period of 49 days was observed. All these clipfish samples showed growth below 4 log cfu/g, with an average of 2.5 ± 1.5 log cfu/g. We have from previous cod studies also seen that HPP can be used to extend the shelf life of fresh cod processed at 500 MPa.

In the saltfish experiment, where different packaging regimes were used, the bacterial load after the rehydration process was 4.0 ± 1.3 log cfu/g. The relatively high standard deviation was due to significant differences ($p<0.001$) in the two rehydration processes, with an average of 2.7 and 5.3 log cfu/g. This shows that it is possible to obtain very low bacterial numbers in rehydrated saltfish and clipfish (2-3 log cfu/g), and this will be a very good starting point and will greatly affect the shelf life of a rehydrated product. Despite the relatively high average bacterial start levels, the HPP combined with different packaging lowered the bacterial numbers to below 2 log cfu/g for some of the treatments. We did not observe any significant difference in packaging among the non-processed rehydrated saltfish samples. After 15 days the average bacterial numbers for saltfish packed with CO_2-emitter and MAP had reached almost 6 log cfu/g, but with relatively high standard deviation indicating that some of the parallel samples were not yet spoiled, Figure 4.

For the HPP-samples, there was no significant difference between the two rehydration batch processes during storage. This indicates that there was a high inactivation of bacteria during HPP, as one of the batches showed high bacterial numbers after the rehydration process. At day 15, there was a significant ($p<0.001$) difference comparing the corresponding packed samples with/without HPP. By evaluating all the storage days together for the HPP samples, vacuum packaging was significantly higher than in MAP or

using CO_2 emitter. There was no significant difference between MAP and CO_2 emitter. The CO_2 emitter samples consisted most likely of an atmosphere close to 100 % CO_2, while for the MAP samples there was decrease/increase in CO_2 and O_2 levels with increasing storage time. This indicates diffusion through the packaging material and may have influenced the bacterial growth in the MAP samples. Looking closer into the data, there was no significant difference between the three packaging regimes at the end of the testing period, at day 49. Attempts to combine different packaging methods and subsequent high-pressure treatment showed little extra effect when packaging with CO_2 present. This is surprising, as we have previously reported a positive synergy effect of combining CO_2 and high pressure (Rode et al., 2015). However, all the HPP-samples showed a bacterial load below 5 log cfu/g (average ~3 log) at day 49, and some individual samples had no growth. This means that all the HPP products had longer shelf life than 49 days. Comparing with the start level at day 0, all HPP-samples had at day 49 a lower amount of bacteria present compared with the bacterial level after rehydration. Based on the results in Figure 2 and 4, it can be summarized that HPP leads to high inactivation of bacteria, and high drip loss. The opposite was seen for the non-pressurised samples; they showed high bacterial growth day 5–15, while they had low drip loss. We do not believe that there is any correlation with higher bacterial numbers leading to lower drip loss.

HPP generally allows products to appear fresh. Depending on the raw material and processing conditions used, changes in texture and appearance may occur, but after cooking many of these changes are not noticeable anymore. Further HPP provides the potential for significantly reduced waste of perishable food. Optimal use of HPP combined with other packaging and packaging technologies (Sterr, Fleckenstein, & Langowski, 2015) will together contribute to new applications and products.

4 Conclusion

Rehydrated clipfish and saltfish can increase shelf life from days to several weeks, depending on the packing and processing conditions. High-pressure processing of rehydrated clipfish and saltfish has proven to provide very long shelf life, at least 49 days can be achieved if processed at 600 MPa for 5 minutes. The drip loss in the HPP samples were somewhat high during storage, but this can possibly be solved with the use of an absorbent in the packages.

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Table 1. *pH measurements in saltfish samples at selected days during the study.*

Packaging	HPP	0	5	15	30	40	49
Air		6.12 ± 0.05					
Vacuum			6.37 ± 0.08	6.28 ± 0.07			
CO_2 emitter			6.21 ± 0.04	6.15 ± 0.06			
MAP			6.06 ± 0.05	6.18 ± 0.05			
Vacuum	x	6.23 ± 0.07		6.36 ± 0.06	6.40 ± 0.07	6.65 ± 0.03	6.33 ± 0.02
CO_2 emitter	x	6.30 ± 0.04		6.11 ± 0.12	6.13 ± 0.07	6.24 ± 0.13	6.11 ± 0.07
MAP	x	6.18 ± 0.01		6.08 ± 0.07	6.20 ± 0.01	6.33 ± 0.06	6.20 ± 0.02

***Table 2**. Colour analysis of clipfish stored for 0, 15 and 49 days. The non-pressurised control (0.1 MPa) was only analysed day 0. The average colour (L*, a*, b*) of the HPP samples (processed at 400, 500 and 600 MPa) are presented for day 0-49.*

Samples	Storage (days)	L*	a*	b*
Control (0.1 MPa)	**0**	74.9 ± 2.8[a]	2.8 ± 1.3[a]	18.0 ± 3.4[a]
HPP samples	**0**	76.3 ± 2.9[a]	2.5 ± 1.5[a]	17.6 ± 4.1[a]
	15	73.1 ± 2.8[b]	4.9 ± 4.2[b]	22.8 ± 2.5[b]
	49	72.7 ± 3.2[b]	5.9 ± 2.6[b]	25.8 ± 2.3[b]

Each colour value in the table is the mean ± standard deviation. The letters (a and b) indicate significant difference ($p < 0.05$) between days of storage for the HPP samples (same column).

Author Statement

Tone Mari Rode; Conceptualization, Validation, Investigation, Writing - Original Draft, Review & Editing

Bjørn Tore Rotabakk; Conceptualization, Validation, Formal analysis, Investigation, Writing - Review & Editing

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Figure 1*. Drip loss (%) of the pressurized (0.1, 400, 500 and 600 MPa) clipfish after processing (day 0) and storage (15 and 49 days). Different superscript within each storage day, indicate significant variation (p<0.05) by GLM and Tukey Method.*

Figure 2*. Drip loss (%) of saltfish after packaging (air, vacuum, CO_2 emitter and MAP) and processing (0.1 and 600 MPa) for a storage period of 49 days. Vac denotes vacuum packaging; MAP: modified atmosphere packaging; Emitter: CO_2 emitter; and HPP: high pressure processing.*

Figure 3*. Bacterial counts in clipfish after processing (0.1, 400, 500 and 600 MPa; day 0) and storage (14 and 49 days), plated on TSA added 2% NaCl.*

Figure 4*. Bacterial counts in rehydrated saltfish after packaging and processing (0.1, 400, 500 and 600 MPa). Sampling were made on different days (day 0-49) and plated on TSA added 2% NaCl. But not all samples were analyzed at all timepoints. At day 0, only samples packed in air (control) were analyzed, in addition to HPP samples. Bacterial counts for samples packed in vacuum (Vac), with CO_2 emitter (Emitter) and modified atmosphere (MAP) are assumed to have the same bacterial count at day 0 as the control.*

Figure 5. *The compression (g) used to press down a 12.7 mm probe A: 20; B: 40; C: 60 and D: 80 % of the sample height of control (0.1 MPa, day 0) and HPP (400, 500 and 600 MPa) clipfish stored for 0, 15 and 49 days.*

Highlights

- High pressure processing (HPP) extends the shelf life of desalted cod
- A shelf life of >49 days can be obtained of HPP desalted cod
- No significant change in color of HPP and control samples after processing
- HPP induced increased driploss

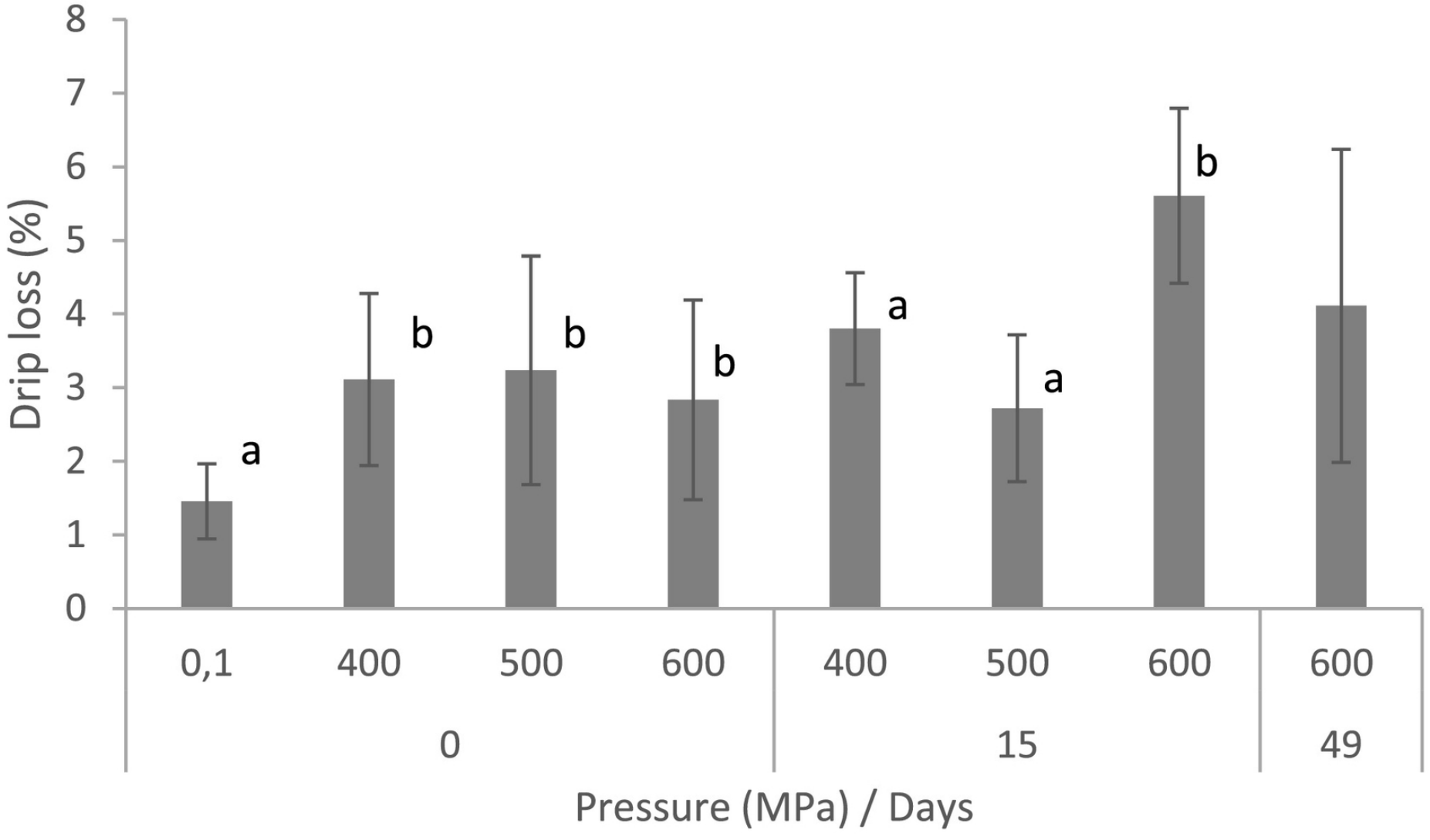

Drip loss (%)
8
7
6
5
4
3
2
1
0
a
b
b
b
a
a
b
0,1
400
500
600
400
500
600
600
0
15
49
Pressure (MPa) / Days

Figure 1

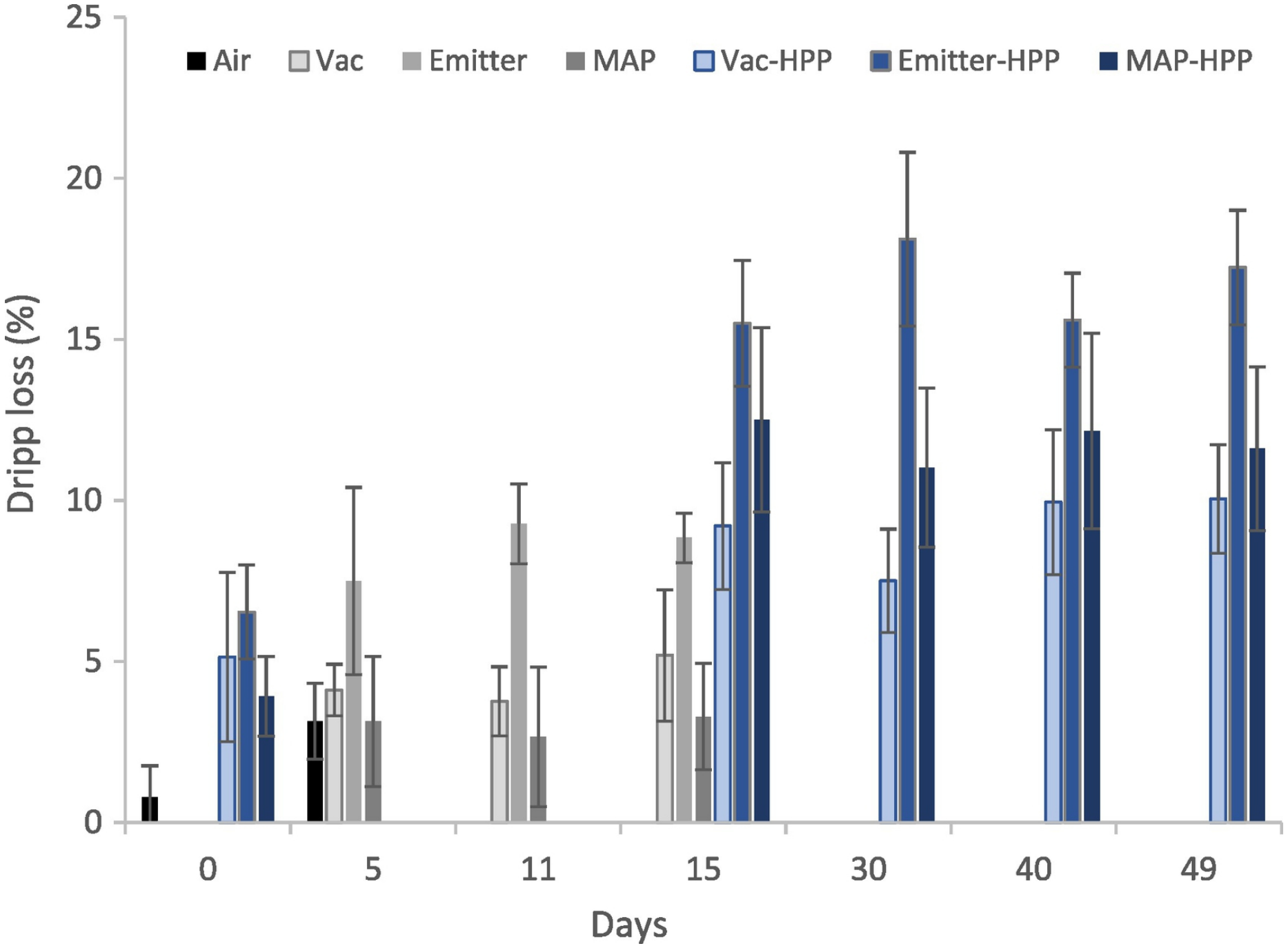

25
20
15
10
5
0
Dripp loss (%)
Air
Vac
Emitter
MAP
Vac-HPP
Emitter-HPP
MAP-HPP
0
5
11
15
30
40
49
Days

Figure 2

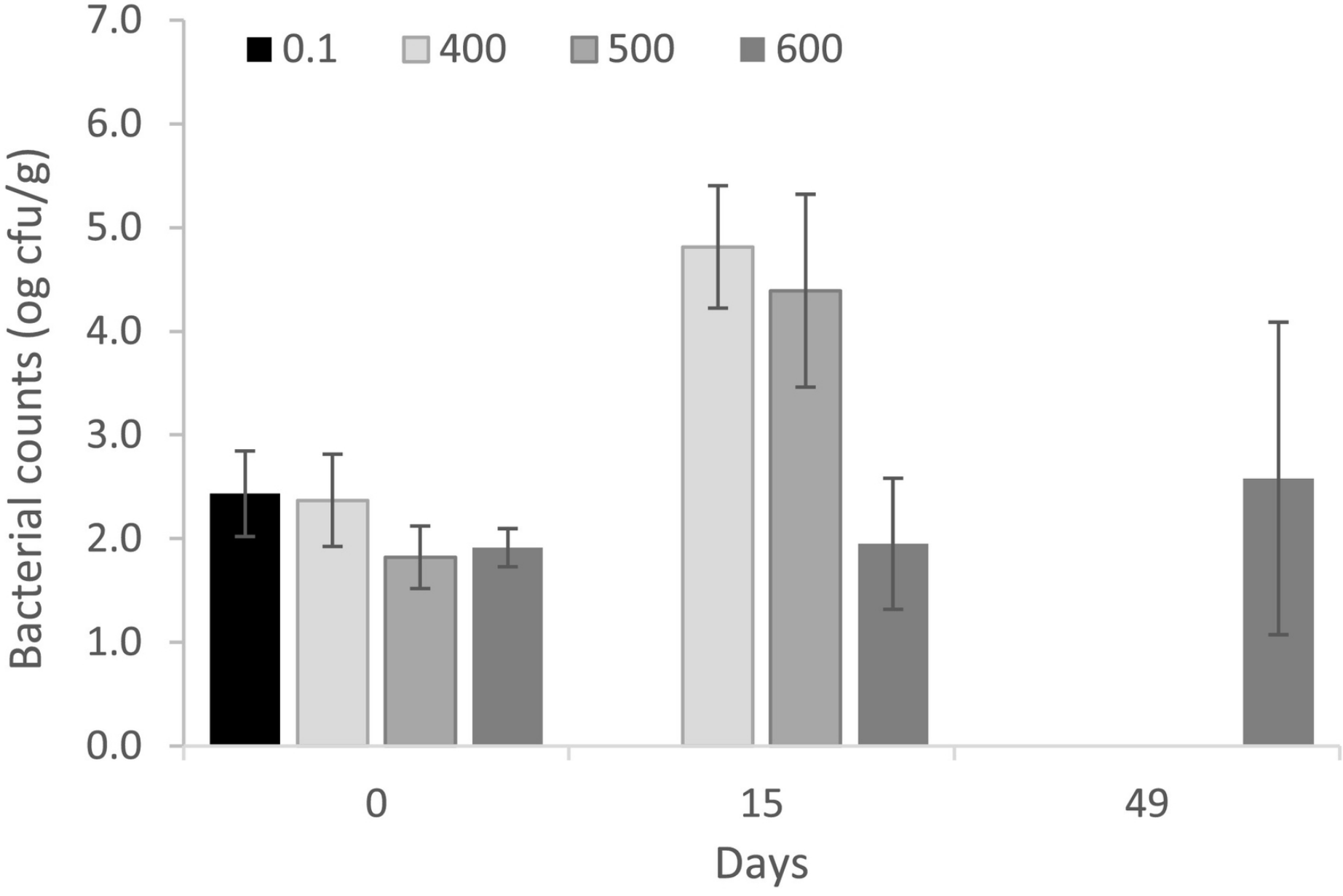

7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.1
400
500
600
Bacterial counts (og cfu/g)
0
15
49
Days

Figure 3

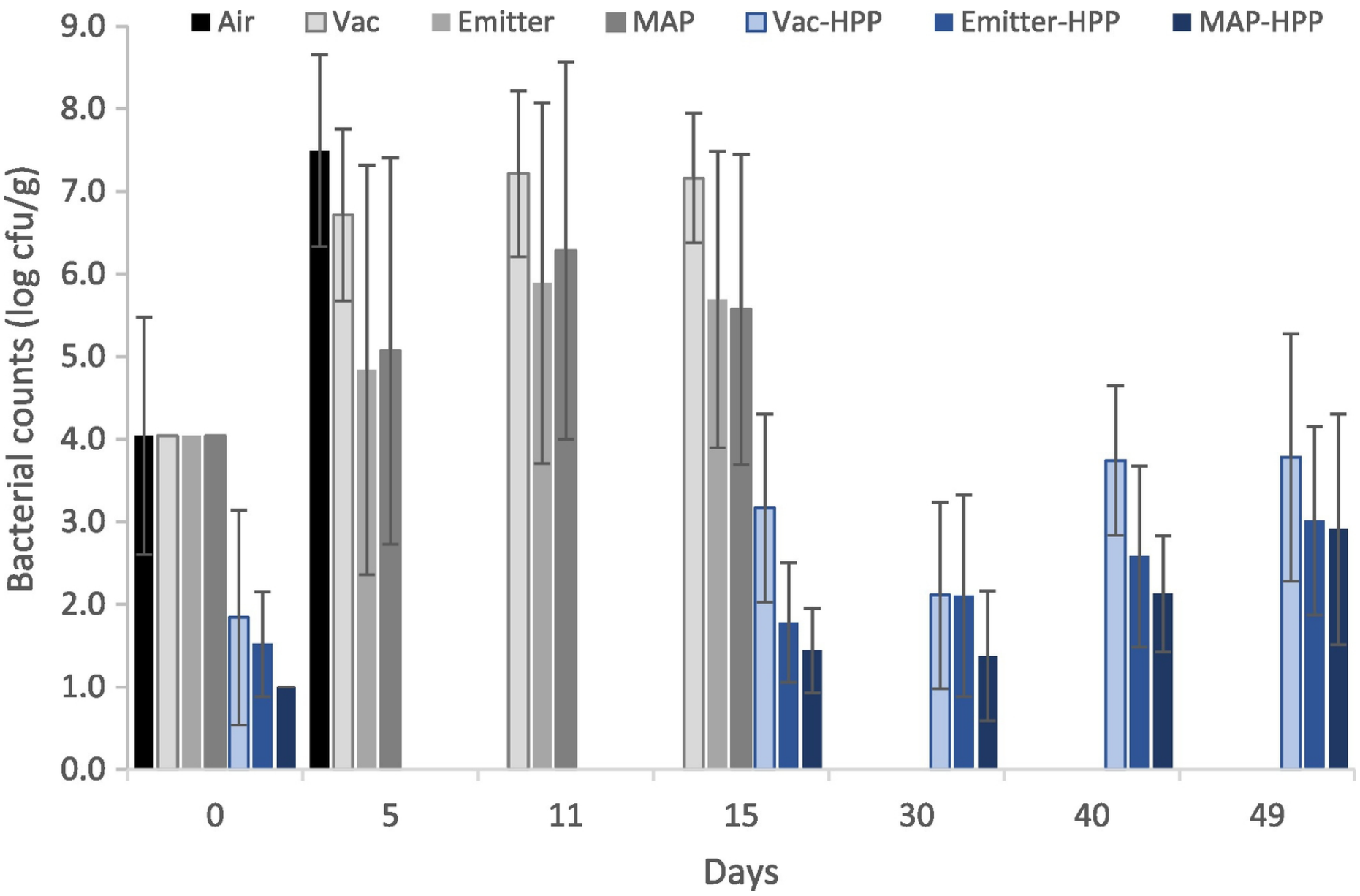
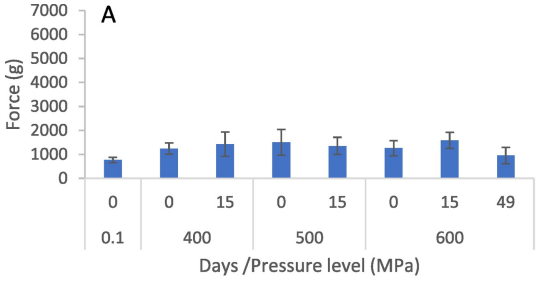
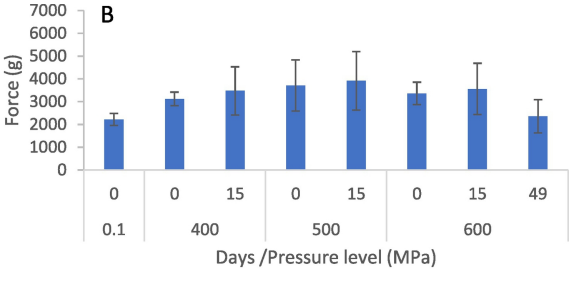
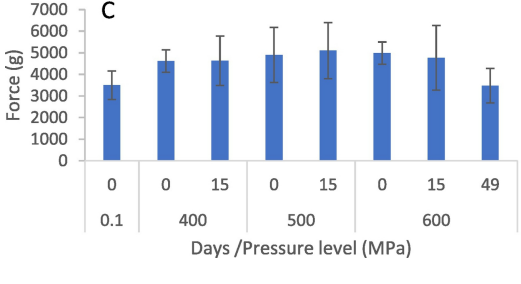

Air
Vac
Emitter
MAP
Vac-HPP
Emitter-HPP
MAP-HPP
Bacterial counts (log cfu/g)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
5
11
15
30
40
49
Days

Figure 4


A
Force (g)
7000
6000
5000
4000
3000
2000
1000
0
0 0 15 0 15 0 15 49
0.1 400 500 600
Days /Pressure level (MPa)


B
Force (g)
7000
6000
5000
4000
3000
2000
1000
0
0 0 15 0 15 0 15 49
0.1 400 500 600
Days /Pressure level (MPa)


C
Force (g)
7000
6000
5000
4000
3000
2000
1000
0
0 0 15 0 15 0 15 49
0.1 400 500 600
Days /Pressure level (MPa)

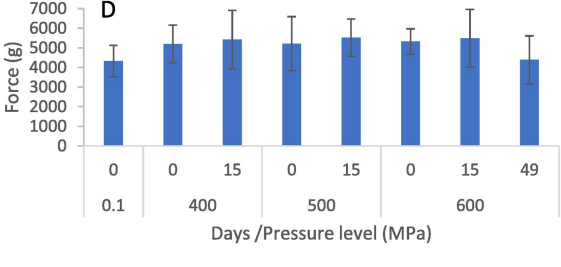

D
Force (g)
7000
6000
5000
4000
3000
2000
1000
0
0 0 15 0 15 0 15 49
0.1 400 500 600
Days /Pressure level (MPa)

Figure 5