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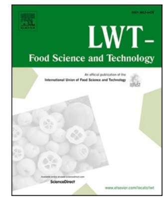

The effect of soluble gas stabilization and high-pressure processing on rehydrated dried salt-cured cod vacuum packaged in bio-based bags.

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ABSTRACT

This study evaluates the combination of soluble gas stabilization (SGS) and high-pressure processing (HPP) as hurdle technology on rehydrated clip fish (dried salt-cured cod) packaged in bio-based materials. The factors studied include SGS/non-SGS, pressure at 400MPa/600 MPa (5 min, 8–9 °C) and vacuum packaging with conventional/bio-based material. Stored over 48 d at 4 °C, the investigated parameters were drip loss, water and protein content, free amino acid (FAA) composition, colour, texture, pH and microbiological shelf life. Results revealed minimal differences in quality between packaging materials. The SGS-treated samples gave higher drip loss, lower water but higher protein and FAA content, and a softer texture. Nevertheless, adding CO_2 prior to HPP significantly extended the product's microbiological shelf life compared to only HPP. Therefore, using a more environmentally friendly bio-based packaging material is feasible while undergoing mild treatments like the combination of SGS and HPP at a lower treatment pressure for shelf-life extension.

1. Introduction

Food packaging is an important process in the food's value chain as it protects the food from physical, chemical, and biological impacts and ensures food safety and security. Around 40% of all plastics produced in the European Union are used in packaging. Of these, around 60% is responsible for food packaging wastes that are often only singly used and discarded (Axelsson-Bakri, Nordenfelt, Ajdič, & Le Nail, 2020). Traditional plastics are often multi-material packaging made from various polymers to enhance good barrier characteristics with distinctive chemical and physical properties. Such plastics are not ideal for recycling and are eventually disposed of in incinerations or landfills (European Commission, 2017). Following the United Nations' Sustainable Development Goals aims, there is an increasing interest in using environmentally friendlier alternatives to traditional plastics, such as mono-layered, bio-based, and biodegradable materials. However, unlike traditional plastics, which have good barriers and mechanical properties to keep the food safe for consumption, such alternatives may not have the same properties, and food spoilage may occur faster. Therefore, there is a trade-off between the choice of alternative packaging versus food quality and safety.

Traditional standard food preservation methods include drying, salting, smoking and fermentation. Dried salt-cured cod, or clip fish (*klippfisk*), is a Norwegian delicacy produced by salting and drying. Norway has a long history of clip fish production, with records dating back to 1640. In the first half of 2023, the export value of clip fish in Norway was 2.5 billion NOK (Norges sjømatråd, 2023). Clip fish in its dried state has a long and stable shelf life of at least one year when stored at low temperatures (Lorentzen, Egeness, Pleym, & Ytterstad, 2016), with a salt and water content of around 20 g/100 g and 50 g/100 g, respectively (Bjørkevoll, Olsen, & Skjerdal, 2003). For consumption, clip fish must be rehydrated to lower the salt content to 2–3 g/100 g. The rehydration process significantly increases water activity and microbial growth. Hence, rehydrated clip fish must be consumed immediately or repackaged to extend its shelf life (Rode & Rotabakk, 2021).

Mild non-thermal processing technologies are gaining increased attention to maintain quality and prolong shelf life without significantly changing the sensory and nutritional characteristics of the food product. Examples of such technologies include high-pressure processing (HPP) and soluble gas stabilization (SGS). Several studies have already established the potential of HPP on aquaculture species for microbiological shelf-life extension while maintaining good nutritional and sensory attributes (Arnaud, de Lamballerie, & Pottier, 2018; Castrica et al., 2021; Cropotova et al., 2020; Lakshmanan, Parkinson, & Piggott, 2007; Rode & Hovda, 2016). The efficiency of HPP depends on the product species, applied pressure, exposure time and temperature (Koutsoumanis et al., 2022). Arnaud et al. (2018) investigated the effects of HPP on frozen and thawed cod and salmon slices and found that performing HPP at 450

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MPa for 5 min at 20 °C was needed to preserve the microbial quality over 2 wk. Nevertheless, colour was affected as the pressure increased over 300 MPa. A recent study by Cropotova et al. (2020) also reported an increase in lightness and softness on haddock and mackerel minces as pressure is increased from 200 to 300 MPa. Hedges and Goodband (2003) also found that HPP on frozen cod fillets at 100 MPa can reduce cook loss. Still, at above 200 MPa, the water holding capacity decreases because of protein denaturation. Therefore, an optimal combination of the process parameters is important.

The high machinery and operational costs of using HPP, especially at high pressure levels (>400 MPa), have limited this technology's commercialized use. Therefore, it can be beneficial to combine other processing technologies with HPP to lower the pressure used (Al-Nehlawi, Guri, Guamis, & Saldo, 2014). SGS is a pre-treatment step before packaging by introducing saturated CO_2 into the product over time. Since CO_2 has bacteriostatic effects, the dissolution of CO_2 into the product's water phase inhibits microbial growth while allowing less use of packaging materials when packaged in a modified atmosphere (Esmaeilian et al., 2021). Studies involving SGS on aquatic food products have shown improved microbiological and sensory shelf life (Abel, Rotabakk, Rustad, Ahlsen, & Lerfall, 2019; de Lima, Rotabakk, Lerin, Monteiro, & Sivertsvik, 2021; Sivertsvik & Birkeland, 2006). In addition, SGS can be combined with thermal and non-thermal processing methods as hurdle technology (Esmaeilian et al., 2021), which is widely used in the food industry by combining a series of methods to prevent the growth of spoilage microorganisms. Limited studies on the combined

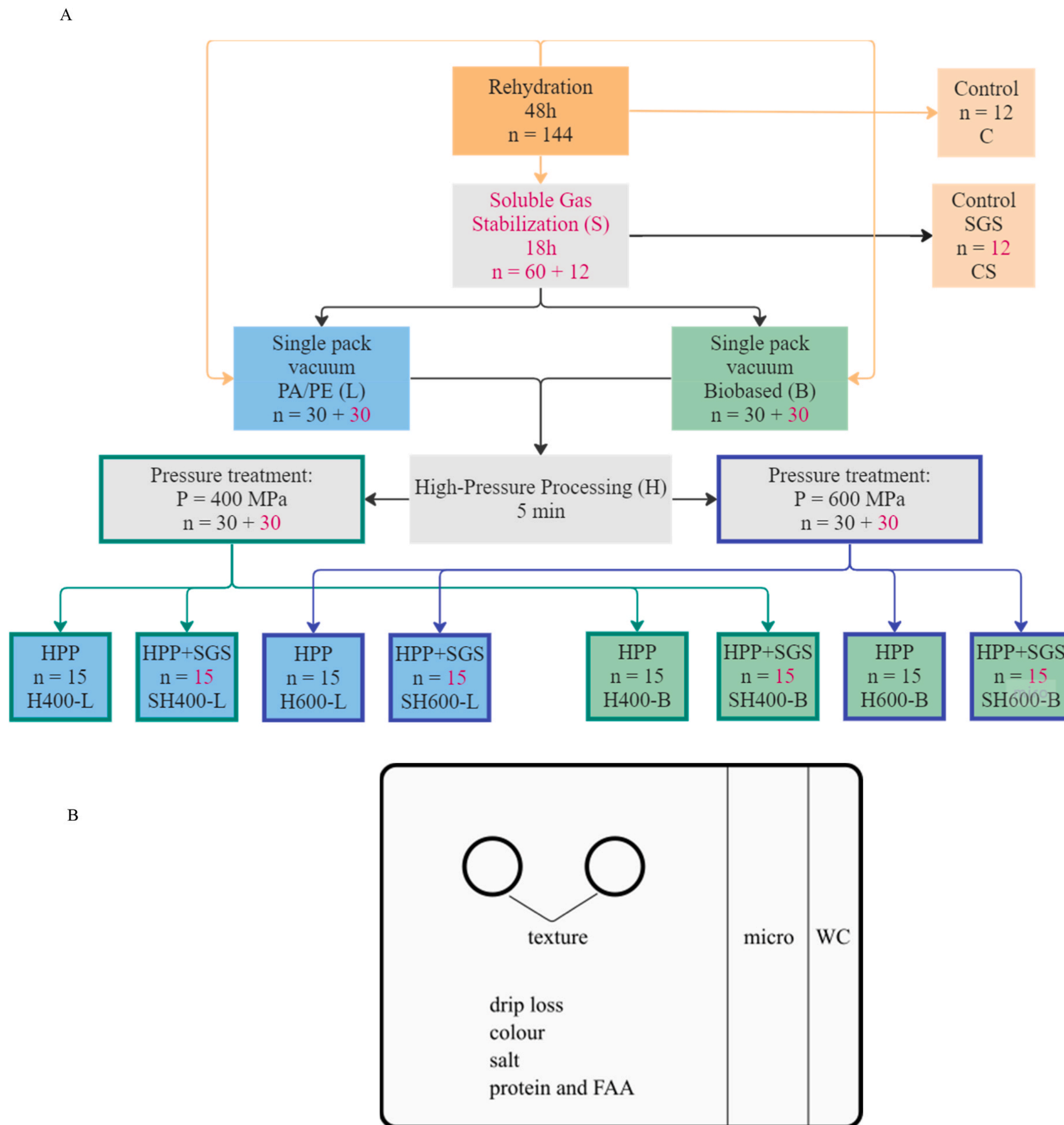

Fig. 1. (a) Experimental set-up of the treatment group for each replicate. After the rehydration step, the clip fish samples were either single packaged or pre-treated with SGS before packaging. The samples were then treated with HPP at 400 or 600 MPa. The red font represents samples treated with soluble gas stabilization (S). The filled colours of the blocks represent the control groups (orange), samples packaged in PA/PE packaging (blue) and samples packaged in BioPBS packaging (green). The colours of the frames represent the pressure level of high-pressure processing at 400 MPa (green) and 600 MPa (blue). (b) A graphical illustration of the quality analyses of each clip fish loin. Drip loss was first measured on the whole loin, then 4–5 g was used for water content (WC) measurements, 10 g for microbiology (micro) analysis, and the rest for colour and texture before freezing the samples for salt, protein and free amino acid (FAA) analyses. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

effect of SGS and HPP have been reported. Rode, Hovda, and Rotabakk (2015) showed that this combination significantly reduced the number of *Listeria innocua* in fish soup samples during storage. Also, Dang, Rode, and Skipnes (2021) found that HPP, with or without SGS, significantly increased the shelf life of pre-cooked chicken breasts. There is a knowledge gap on the synergistic combination of SGS and HPP as hurdle technology, with bio-based food packaging. Therefore, this study aims to investigate the efficacy of this combination to evaluate the quality and shelf life of rehydrated clip fish.

2. Materials and methods

Before this experiment, a preliminary study was carried out on clip fish, SGS and non-SGS treated, and then exposed to either 300 MPa, 400 MPa, or 600 MPa for 5 min at 8–9 °C. These clip fish were vacuum packaged in only PA/PE bags. The results (data not shown) concluded that clip fish exposed to 400 MPa and 600 MPa gave the most promising results regarding microbiological shelf life. Therefore, these two pressures were chosen for this study.

2.1. Rehydration

This three-factorial study was carried out twice with three parallels per treatment group. The following variables were studied: SGS (SH: with SGS/H: without SGS), processing pressure (400 MPa/600 MPa), and type of packaging material (L: PA/PE/B: bio-based). Two treatment groups served as controls, with 12 samples per group (CS: with SGS/C: without SGS), giving a total of 10 treatment groups. Fig. 1a illustrates the experimental setup and the respective abbreviations of the groups.

A total of 288 skinned and deboned clip fish loins (n = 144/replicate) were obtained from a local supplier in Norway. Rehydration was carried out with pre-cooled water using the method described by Rode and Rotabakk (2021). The total rehydration time was 48 h, with water drained and replaced after 6 h, 12 h and 24 h. For each replicate, eight clip fish samples were tagged and weighed at each time point to monitor the rehydration process.

2.2. Processing

2.2.1. Soluble gas stabilization

Half of the rehydrated clip fish (n = 72) were randomly chosen and underwent the SGS treatment. These loins were distributed in batches in 5 steel grates placed in PA/PE sous vide bags (450 × 700 mm, NorEngros, Norway). The bags were exposed to excess CO_2 using a vacuum chamber machine (Webomatic, Bochum, Germany) connected to 100 % CO_2, then sealed and stored in a cold room at 4 °C for 12 h. Meanwhile, the other half of the rehydrated clip fish unexposed to SGS were packaged in air. After 12 h, the headspace gas composition for those exposed to SGS was measured using a PBI Dansensor CheckMate 9900 Headspace Gas Analyser (Nordic Supply System, Norway) and then repackaged.

2.2.2. Packaging

All samples were marked, weighed, and repackaged in 99% vacuum (Webomatic, Bochum, Germany) in either a PA/PE sous vide bag (n = 60) or a high barrier bio-based bag made from cellulose film laminated to Bio-Polybutylene succinate (BioPBS) (n = 60). Both control groups were only vacuum packaged in the sous vide bags. The samples were kept in a cold room at 4 °C before further treatment with HPP the next day.

According to technical information, the multi-layered PA/PE sous vide bags (180 × 140 mm, Lietpak, Vilnius, Lithuania) have a thickness of 80 ± 5% μm with sealing temperature from 140 to 160 °C, an oxygen transmission rate (OTR) and a water vapour transmission rate (WVTR) of ~52 cc/m^2/24 h (23 °C, 75% RH) and 2.3 cc/m^2/24 h, respectively. The bio-based packages (250 × 160 mm, Grounded Packaging, Sydney, Australia) have a thickness of 44.1–53.9 μm and an OTR and WVTR of <1 cc/m^2/24 h (23 °C, 0% RH) and <14 g/m^2/24 h (38 °C, 90% RH), respectively.

2.2.3. High-pressure processing

Classified as day 0, rehydrated clip fish subjected to HPP were pressurized at either 400 MPa (n = 60) or 600 MPa (n = 60) for 5 min at 8–9 °C using a high hydrostatic pressure machine QFP 2L-700 (Avure Technologies Inc., Columbus, USA). The come-up time was approximately 65 and 100 s for 400 and 600 MPa, respectively, and the pressure release was immediate. After HPP, samples were placed in ice water for rapid chilling before cold storage. All samples were then stored in a cold room at 4 °C.

2.3. Quality analysis

Quality analyses were done periodically on random samples, including drip loss, water, salt, protein content, free amino acid (FAA) composition, colour, texture, pH and microbiology (n = 3/group). The control groups were analyzed on days 0, 3 and 6, and additionally on day 10 for those treated with SGS. Otherwise, the HPP-treated samples were analyzed at days 0, 13, 24, 34 and 48. Each clip fish loin was divided into 3 pieces for water content, microbiological analysis and the rest for colour and texture measurements before freezing at −80 °C for further analysis of salt and protein, and FAA content. Fig. 1b shows an illustration of where sampling was done on each loin.

2.3.1. Drip loss, water and salt content

Drip loss was weighed on each sampling day immediately after opening the packages, calculated by the % difference to its initial weight. Around 5 g of the sample was cut for water content analysis, following the gravimetric drying method at 105 °C for 16–18 h (ISO 6496, 1999). Salt content (g/100 g NaCl) was measured on samples from day 0 using the Mohr's titration method with $AgNO_3$ using an Easy Cl automatic titrator (Mettler Toledo, Norway). Frozen rehydrated clip fish samples were thawed, and around 500 mg were cut from the middle portion of the loin and homogenized with 100 mL distilled water (55 °C) using an Ultra Turrax T25 (Janke & Kunkel IKA, Labortechnik, Staufen, Germany) at 13 500 rpm for 40s. One mL 1 M HNO_3 was added to the homogenized solution before titration, and titration stopped once the equivalence point of AgCl was formed.

2.3.2. Protein content and free amino acid

Frozen samples from days 0, 24 and 48 were homogenized using a Braun MultiQuick 5 Vario hand blender. Approximately 1–1.5 g of the homogenized sample was used to determine the protein content, which was calculated from the nitrogen content obtained from the Kjeldahl method (NMKL, 2003). The rest of the homogenized samples were used to determine the FAA profile using the method by Osnes and Mohr (1985). A water-soluble protein extract was made by re-homogenizing 2 g of the samples containing 10 mL distilled water at 14 500 rpm for 50 s. The solution was centrifuged at 7000 rpm for 4 min at 4 °C (Kubota, Model 1700, Tokyo, Japan). 1 mL of the soluble protein extract was transferred to a 1.5 mL Eppendorf tube and mixed with 0.25 mL 10% sulfosalicylic acid (Merck Millipore, Darmstadt, Germany). The tubes were stored at 4 °C for at least 30 min before centrifuging at 10 000 rpm for 10 min at 4 °C (ThermoFisher, Megafuge 8 R Centrifuge, Waltham, USA). Afterwards, the supernatant was collected, diluted to 1:25 with deionized water, and filtered through a 0.22 μm polyethersulfone membrane filter (VWR International, USA) into 1.5 mL HPLC vials. The FAA profile was analyzed using an ultra HPLC (UltiMate 300, Thermo Scientific) equipped with a TSP P400 pump, ultimate 3000WP injector, RF2000 detector and a Nova-Pak C18 column (WAT086344, particle size: 4 μm, 3.9 mm × 150 mm, Waters Corp., USA). Methanol and sodium acetate (0.08 M, Alfa Aesar, Haverhill, USA) with 2% tetrahydrofuran (Merck Millipore, Darmstadt, Germany) were used as mobile phases. The flow rate was adjusted to 0.9 mL/min (Kendler et al., 2023).

2.3.3. Surface colour and texture

The surface colour of the samples was measured using a DigiEye® full system (VeriVide Ltd., Leicester, UK) connected to a digital camera (Nikon D80, 35 mm lens, Nikon Corp., Japan). The system was calibrated on each sampling day. Digipix software (v2.8, VeriVide Ltd., Leicester, UK) was used to analyze the colour parameters L*, a* and b* values, where L* represents lightness ($L^* = 0 =$ white, $L^* = 100 =$ black), a* represents redness ($a^* > 0$) and b* represents yellowness ($b^* > 0$).

The texture of the samples was analyzed with a TA.XT® plus texture analyzer (Stable Micro Systems, Godalming, UK) equipped with a 50 kg load and a flat-end cylindrical probe (12.7 mm P/0.5). The probe was pressed down at a rate of 2 mm/s to 60% of the sample height, describing the firmness of the loin. Analysis was done in replicates. The Texture Exponent software recorded the force-time graph (version 8.0.16.0, Stable Micro Systems, Godalming, UK).

2.3.4. Microbiology and pH

Microbiological analysis was carried out to quantify total psychrotrophic counts (PC), total aerobic plate counts (APC) and hydrogen sulphide-producing bacteria (HSPB) ($n = 3$/group), according to the NMKL method No. 184 (NMKL, 2006). Around 10 g of muscle samples were cut and diluted to 1:9 with sterile peptone water, then homogenized for 2 min using a Stomacher 400 Laboratory Blender (Seward Medical, London, UK). About 50 mL of the stomacher solution was transferred to sterile Falcon tubes for pH measurement. The pH was measured at room temperature with a pH meter (Mettler Toledo FiveEasy Plus with LE410 electrode, Mettler Toledo, Norway).

Appropriate dilution series were made from the stomacher solution with sterile peptone water. 1 mL from the dilution was transferred to petri dishes before 10–12 mL of sterile iron agar containing L-cysteine was poured over. 49.2 μL of the dilution was mechanically plated to Long and Hammer (L&H) agar plates using a spiral plater (EddyJet, IUL Instruments, Barcelona, Spain). Some manual plating was done at low dilutions. The iron agar and L&H agar plates were incubated at 25 °C for 72 ± 6 h and 15 °C for 5–7 d, respectively. The total colonies from L&H were quantified for PC, while the total and black colonies from iron agar were quantified for APC and HSPB, respectively. Microbial concentrations are expressed as log cfu/g.

2.4. Statistical analysis

Statistical analysis was performed in IBM SPSS Statistics 28.0.1.0. A *t*-test on independent samples was first used to determine any significant differences between replicates 1 and 2 for all the data obtained from quality analyses. As there were no differences ($p > 0.05$), the data from replicates 1 and 2 were combined and treated as one data set. In addition, the control groups were excluded from the statistical analysis since they were neither treated with HPP nor packaged in bio-based packages.

The statistical methods used include general linear modelling (GLM), analysis of variance (one-way ANOVA) and post hoc Tukey's HSD test if there were significant differences. Moreover, Pearson's correlations were conducted between the different parameters. For GLM, SGS (with SGS, without SGS), processing pressure (400 MPa, 600 MPa) and packaging material (L = PA/PE, B = BioPBS) were set as fixed factors. The sample fillet height was included as a covariate during statistical analysis of salt content and textural properties. A principal component (PCA) was performed in Unscrambler 11 to visualize the FAA data better. All results are reported as mean ± standard error of the mean. The α-value was set to 0.05.

3. Results and discussion

3.1. Headspace gas composition

The resulting headspace gas composition after SGS treatment gave a significant difference between replicates 1 and 2 ($p < 0.008$), where replicate 1 had a 91.7 ± 1.6% CO_2 and 1.7 ± 0.3% O_2 and replicate 2 had 99.4 ± 0.1% CO_2 and 0.5 ± 0.03% O_2. Even so, statistical analysis did not show significant differences between the results from both replicates and were hence treated as one.

3.2. Weight changes and drip loss

The highest weight increase during rehydration occurred in the first 6 h by 9.1 ± 1.8%, due to the greatest concentration difference between salt and water. Afterwards, the weight increased by 3.2 ± 1.1% and 3.8 ± 0.5% after 12 h and 24 h, respectively. The resulting total weight increase was 17.0 ± 0.7% after 48 h. These results aligned with the study of Rode and Rotabakk (2021), with an increase of 17.7 ± 1.1% after 48 h rehydration.

Drip loss significantly increased through storage ($p < 0.001$), an expected result since drip loss is a time-dependent process. A general observation was seen with a higher drip loss for SGS treated samples ($p < 0.001$). There were no differences between the packaging materials ($p = 0.290$) or the pressure levels ($p = 0.280$). Fig. 2 illustrates the drip loss of samples after processing. On day 0, the control group C and SH600-L gave the lowest and highest drip loss, respectively. There were no significant differences between the control groups ($p = 0.055$). The large variations within samples could likely be due to the differences in loin size, as there was a negative correlation between drip loss and fillet height ($r = -0.438$, $p < 0.001$). Rode and Rotabakk (2021) reported a greater drip loss for HPP-treated clip fish than those untreated due to denaturation of myosin and fluid release. CO_2, as a weak acid, can also influence water holding capacity by lowering the pH, thereby increasing drip loss (Sivertsvik, 2007).

3.3. Salt, water and protein content

There were no significant differences in salt content among the treatment groups on day 0 ($p = 0.099$). The overall salt content obtained was 3.3 ± 0.2 g/100 g. As expected, a thicker fillet resulted in a higher salt content ($r = 0.614$, $p < 0.001$). Salt content was negatively correlated with drip loss ($r = -0.445$), while positively correlated with firmness ($r = 0.472$). This may be attributed by the interaction of salt with the muscle proteins that increases hydration and improves the water holding capacity.

On day 0, the water content after rehydration measured in the control groups was 79.5 ± 0.32% (group C) and 79.6 ± 0.61% (group CS), while the protein content was 18.1 ± 1.95% (group C) and 18.4 ± 0.93% (group CS). These values are similar to the water and protein content of fresh cod, estimated to be between 78 and 83% and around 20%, respectively (Huss, 1995).

Table 1 shows the water and protein content measurements for all the treated groups. Water and protein content had a negative correlation ($r = -0.474$). The type of packaging material only significantly influenced the water content for H400-L compared to H400–B on day 0 ($p = 0.006$). Otherwise, the packaging material did not significantly influence water ($p = 0.859$) or protein content ($p = 0.169$). Through storage, the greatest decrease in water content occurred between days 0 and 13 for all groups ($p < 0.001$). Afterwards, the water content remained somewhat stable. Samples that underwent the SGS treatment also generally gave a lower water content ($p < 0.001$) but higher protein content ($p < 0.001$) than those without. Samples treated at 600 MPa also had a slightly lower water content ($p = 0.008$) and higher protein content ($p = 0.001$). Hedges and Goodband (2003) found that cod treated at 100 MPa gave a lower cook loss than the untreated control, and Lakshmanan et al. (2007) found that HPP at 200 MPa caused an increase in water content on fresh and cold smoked salmon. The rationale behind this observation was possibly due to the enhancement of the hydration capacity of proteins, in particular the myosin tail and actin. Nevertheless, this does not directly imply an increase in water holding

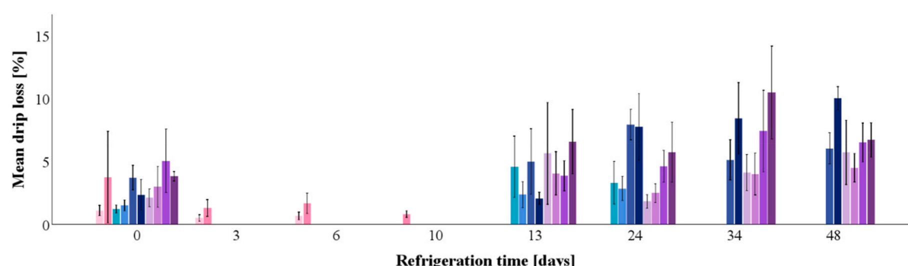

Fig. 2. Mean drip loss (%) of samples on through 48 days storage (GLM excluding control groups; SGS treatment: $p < 0.001$; pressure: $p = 0.280$; storage day: $p < 0.001$; packaging material: $p = 0.290$). The coloured bars represent groups ■ C, ■ CS, ■ H400-L, ■ H400–B, ■ SH400-L, ■ SH400–B, ■ H600-L, ■ H600–B, ■ SH600-L and ■ SH600–B.

Table 1

Water and protein content measurement of all treatment groups. "H" and "SH" denote processing with high-pressure or soluble gas stabilization and high-pressure processing, respectively. "400" and "600" represent high-pressure processing at 400 MPa or 600 MPa, respectively. "L" and "B" denote the PA/PE or BioPBS packaging materials, respectively.

Water content (%)									
Group	H400		SH400		H600		SH600		p-value[1]
Day/Package	L	B	L	B	L	B	L	B	
0	**78.1 ± 0.67**[ab]	**79.7 ± 0.50**[d]	79.2 ± 0.21[bcd]	78.6 ± 0.41[abc]	79.8 ± 0.26[d]	79.5 ± 0.40[cd]	77.7 ± 0.37[a]	78.1 ± 0.46[ab]	$p_D < 0.001$*
13	79.0 ± 0.32[d]	78.1 ± 0.33[bcd]	77.5 ± 0.43[ab]	76.6 ± 0.15[a]	78.7 ± 0.47[cd]	77.8 ± 0.55[bcd]	76.4 ± 0.44[a]	77.6 ± 0.45[abc]	$p_{pr} = 0.008$*
24	78.3 ± 0.68[b]	78.1 ± 0.08[b]	77.2 ± 0.33[ab]	77.9 ± 0.55[ab]	77.7 ± 0.36[ab]	77.3 ± 0.37[ab]	76.8 ± 0.24[a]	77.6 ± 0.28[ab]	$p_T < 0.001$*
34			77.6 ± 0.25[b]	77.3 ± 0.23[ab]	77.5 ± 0.13[ab]	76.8 ± 0.07[ab]	76.4 ± 0.59[a]	76.9 ± 0.31[ab]	$p_{pa} = 0.859$
48			77.4 ± 0.46[ab]	78.1 ± 0.44[b]	77.8 ± 0.50[b]	77.5 ± 0.24[ab]	77.2 ± 0.45[ab]	76.4 ± 0.27[a]	
Protein content (%)									
Group	H400		SH400		H600		SH600		p-value[1]
Day/Package	L	B	L	B	L	B	L	B	
0	18.4 ± 0.34[a]	18.8 ± 0.36[abc]	18.5 ± 0.23[ab]	18.6 ± 0.25[ab]	18.0 ± 0.55[a]	18.6 ± 0.15[ab]	19.4 ± 0.32[c]	19.3 ± 0.20[bc]	$p_D = 0.476$
24	17.9 ± 0.08[a]	18.5 ± 0.31[ab]	18.5 ± 0.25[ab]	19.0 ± 0.10[bc]	19.1 ± 0.30[bc]	18.6 ± 0.40[ab]	19.7 ± 0.15[c]	19.5 ± 0.21[c]	$p_{pr} = 0.001$*
48			18.8 ± 0.19[a]	19.1 ± 0.31[ab]	18.6 ± 0.39[a]	18.4 ± 0.35[a]	19.2 ± 0.27[ab]	19.8 ± 0.26[b]	$p_T < 0.001$*
									$p_{pa} = 0.169$

Bold text highlights significant differences ($p < 0.05$) pairwise between packaging materials within the same treatment and storage day.

[1] p-values generated using general linear model (GLM) where p_D, p_{pr}, p_T, and p_{pa} represent storage day, pressure, SGS treatment and packaging.

[*] statistically significant when $p < 0.05$.

[abcd] highlights significant differences between treatment groups on the same day.

capacity. The lower water content observed in this study could be related to the use of higher pressure. Fiber compression can be observed at pressures >300 MPa, which reduces water holding capacity and increases drip loss, leading to a reduction in water content (Oliveira, Neto, Santos, Ferreira, & Rosenthal, 2017).

3.4. Free amino acid profile

Fig. 3a illustrates the correlation loading plot for the examined FAAs while Fig. 3b to d shows the score plots of the distribution based on storage days, SGS treatment and HPP pressure. The first two PCAs explained 99% of the total variances of the samples and FAA variables. Amino acids in their free form are associated with the perception of taste and contribute to the overall flavour in foods. FAAs related to sweetness include alanine (ala), glycine(gly)/arginine(arg), threonine (thr) and serine (ser) while those related to bitterness include methionine (met), histidine (his), lysine (lys), tyrosine (tyr), leucine (leu), isoleucine (ile) and valine (val). Lastly, FAAs related to acidic or umami flavour include aspartic acid (asp) and glutamic acid (glu) (Delompré, Guichard, Briand, & Salles, 2019; Song et al., 2020). As observed, all FAAs are closely clustered together on the outer eclipse and followed along PC1. In addition, they are also positively correlated to each other. Asparagine (asn) is an amino acid not usually related to flavour and followed along PC2. There was no grouping for packaging material (data not shown), indicating that this did not significantly influence the FAAs and was therefore not presented in the score plot. The grouping of FAA depended on increasing storage time (Fig. 3b) and the presence of CO_2 (Fig. 3c). From Fig. 3d, HPP treatment at 400 MPa seemed to affect the amount of FAAs. The results suggest that with HPP, proteolysis occurred causing the release of FAAs through time and contributing to off-flavours and taste. Zhang, Bi, Wang, Cheng, and Chen (2019) reported that HPP treatment in cod proteins induced protein denaturation and enhance solubility. In addition, CO_2 could have contributed to protein dissociation. Since CO_2 is soluble in the muscle, the dissolution of CO_2 leads to carbonic acid formation, which may have contributed to the acidic flavour and caused a decrease in pH. This was confirmed by the correlation analysis where pH was negatively correlated to FAAs related to sweetness ($r = -0.498$), bitterness ($r = -0.528$) and acidic flavour ($r = -0.525$).

3.5. Colour and texture

On day 0, the colour of the control groups was: Group C (L*: 95.0 ± 1.46; a*: 0.1 ± 0.45; b*: 12.7 ± 0.85) and group CS (L*: 92.6 ± 2.08, a*:1.1 ± 0.67, b*:13.3 ± 1.10). Table 2 shows the colour parameters of the treated groups, which changed during the whole storage period (Table 2, $p < 0.001$). The pressure level ($p = 0.049$) and packaging material ($p = 0.017$) significantly affected yellowness. An actual variation in packaging material was only observed on day 13. Those packaged in BioPBS (H400–B) gave a more yellowish colour than PA/PE

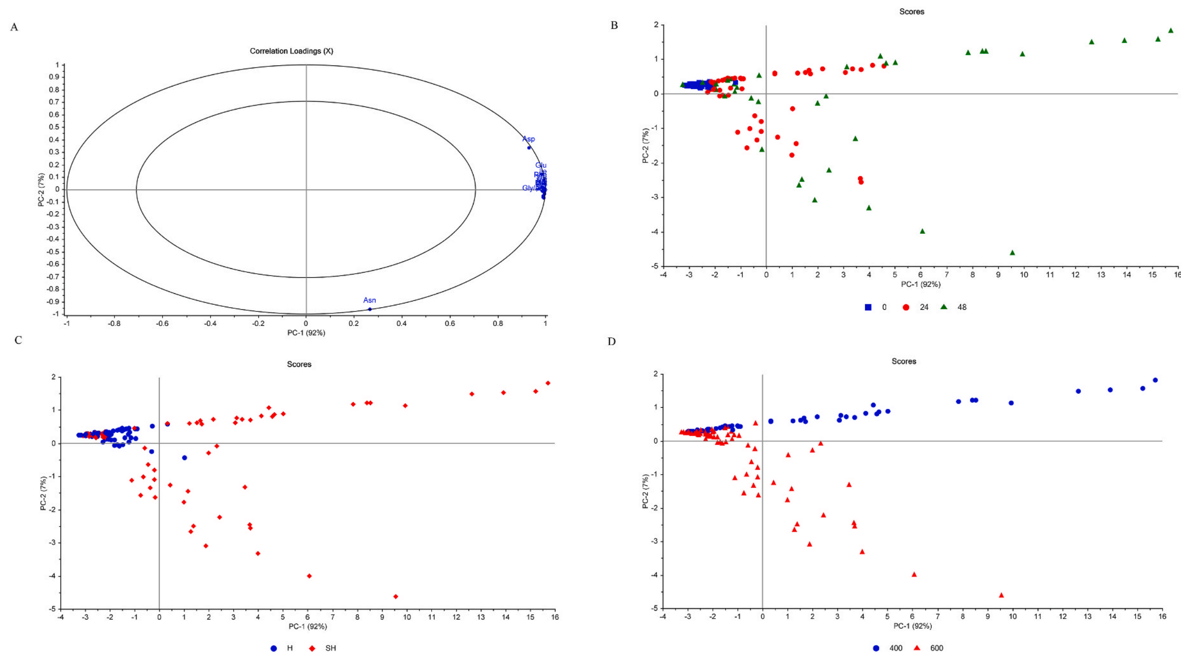

Fig. 3. (a) Correlation loading plot. The inner and outer eclipse represent 50% and 100% of the explained variance, respectively; (b) Score plots categorised based on storage days 0, 24 and 48, (c) SGS treatment (H: without SGS/SH: with SGS), and (d) HPP treatment pressure (400 MPa/600 MPa).

Table 2
Surface colour measurements, L*, a* and b* of all treatment groups (n = 6/group). "H" and "SH" denote processing with high-pressure or soluble gas stabilization and high-pressure processing, respectively. "400" and "600" represent high-pressure processing at 400 MPa or 600 MPa, respectively. "L" and "B" denote the PA/PE or BioPBS packaging materials, respectively.

Lightness, L*									
Group	H400		SH400		H600		SH600		p-value[1]
Day/Package	L	B	L	B	L	B	L	B	
0	94.9 ± 0.37	94.5 ± 0.23	95.0 ± 0.66	95.6 ± 0.92	95.5 ± 0.98	94.1 ± 1.11	94.7 ± 0.29	94.1 ± 1.24	$p_D < 0.001$*
13	**92.2 ± 0.82**[a]	**94.4 ± 0.76**[bc]	93.1 ± 0.94[ab]	94.8 ± 1.27[bc]	94.8 ± 0.58[bc]	95.6 ± 0.44[bc]	94.7 ± 0.11[bc]	95.8 ± 0.55[c]	$p_{pr} = 0.727$
24	96.3 ± 0.95[c]	96.3 ± 0.51[c]	96.7 ± 0.39[c]	96.7 ± 0.84[c]	94.1 ± 1.02[ab]	96.1 ± 0.35[abc]	**94.0 ± 0.64**[a]	**96.1 ± 0.62**[bc]	$p_T = 0.604$
34			93.1 ± 0.38[a]	92.6 ± 0.32[a]	**95.7 ± 1.36**[b]	**92.1 ± 0.38**[a]	92.8 ± 1.43[a]	93.8 ± 0.53[ab]	$p_{pa} = 0.808$
48			95.0 ± 0.31	94.6 ± 0.41	94.9 ± 0.49	94.6 ± 0.63	95.1 ± 0.33	94.5 ± 0.60	
Redness, a*									
Group	H400		SH400		H600		SH600		p-value[1]
Day/Package	L	B	L	B	L	B	L	B	
0	−0.0 ± 0.08[ab]	−0.3 ± 0.16[a]	−0.2 ± 0.09[ab]	0.2 ± 0.22[b]	−0.5 ± 0.09[a]	−0.1 ± 0.09[ab]	−0.3 ± 0.10[a]	−0.0 ± 0.13[ab]	$p_D < 0.001$*
13	−0.3 ± 0.17[ab]	−0.2 ± 0.13[ab]	0.2 ± 0.25[b]	−0.1 ± 0.23[ab]	−0.5 ± 0.14[a]	−0.4 ± 0.16[a]	−0.2 ± 0.07[ab]	−0.4 ± 0.21[a]	$p_{pr} = 0.782$
24	−0.4 ± 0.32[ab]	−0.5 ± 0.16[ab]	−0.4 ± 0.22[ab]	−0.8 ± 0.13[a]	−0.1 ± 0.18[bc]	−0.2 ± 0.11[bc]	**0.3 ± 0.08**[c]	**−0.3 ± 0.29**[b]	$p_T = 0.099$
34			0.2 ± 0.16[b]	−0.0 ± 0.12[ab]	**−0.4 ± 0.28**[a]	**0.4 ± 0.34**[b]	0.4 ± 0.25[b]	−0.0 ± 0.05[ab]	$p_{pa} = 0.392$
48			−0.4 ± 0.12	−0.6 ± 0.05	−0.4 ± 0.03	−0.5 ± 0.13	−0.3 ± 0.07	−0.5 ± 0.14	
Yellowness, b*									
Group	H400		SH400		H600		SH600		p-value[1]
Day/Package	L	B	L	B	L	B	L	B	
0	11.2 ± 0.35[ab]	11.6 ± 0.35[b]	10.5 ± 0.30[ab]	11.8 ± 0.64[b]	9.6 ± 0.37[a]	11.1 ± 0.28[ab]	10.7 ± 0.65[ab]	12.2 ± 0.24[b]	$p_D < 0.001$*
13	**11.4 ± 0.10**[a]	**13.3 ± 0.79**[b]	12.2 ± 0.52[ab]	12.2 ± 0.58[ab]	11.5 ± 0.30[a]	11.8 ± 0.25[ab]	11.7 ± 0.55[ab]	12.4 ± 0.35[ab]	$p_{pr} = 0.049$
24	12.4 ± 1.10	11.4 ± 0.37	11.7 ± 0.32	11.0 ± 0.37	12.6 ± 0.81	12.0 ± 0.68	12.4 ± 0.23	11.2 ± 0.58	$p_T = 0.944$
34			13.6 ± 0.82[c]	13.3 ± 0.72[bc]	11.1 ± 0.65[a]	12.7 ± 0.40[abc]	11.6 ± 0.26[ab]	12.4 ± 0.29[abc]	$p_{pa} = 0.017$
48			11.5 ± 0.63[ab]	13.0 ± 1.13[b]	11.2 ± 1.15[a]	11.3 ± 1.16[ab]	11.2 ± 0.45[a]	12.2 ± 0.45[ab]	

Bold text highlights significant differences ($p < 0.05$) pairwise between packaging materials within the same treatment and storage day.
[1] p-values generated using general linear model (GLM) where p_D, p_{pr}, p_T, and p_{pa} represent storage day, pressure, SGS treatment and packaging.
[*] statistically significant when $p < 0.05$.
[abcd] highlights significant differences between treatment groups on the same day.

(H400-L, $p = 0.048$). Otherwise, there were no differences between pressure, SGS exposure and packaging material. Similar to our results, Rode and Rotabakk (2021) reported no significant differences between the colour for untreated clip fish and the clip fish treated at 500 MPa.

Fig. 4 presents the textural analysis results. On day 0, samples subjected to the mild treatment processes were harder and firmer than those of the control groups. The packaging material did not influence the breaking force ($p = 0.935$) and firmness ($p = 0.194$). There were also no differences between storage days in all treated groups (breaking force: $p = 0.092$; firmness: $p = 0.129$). The minimal differences observed in colour and textural properties during storage may be ascribed to the already denatured proteins during the drying and salting of clip fish before the rehydration process.

Samples treated with SGS (breaking force: $p < 0.001$; firmness: $p = 0.002$) at a lower pressure of 400 MPa (breaking force: $p < 0.001$; firmness: $p = 0.006$) generally gave a softer texture. Several research on

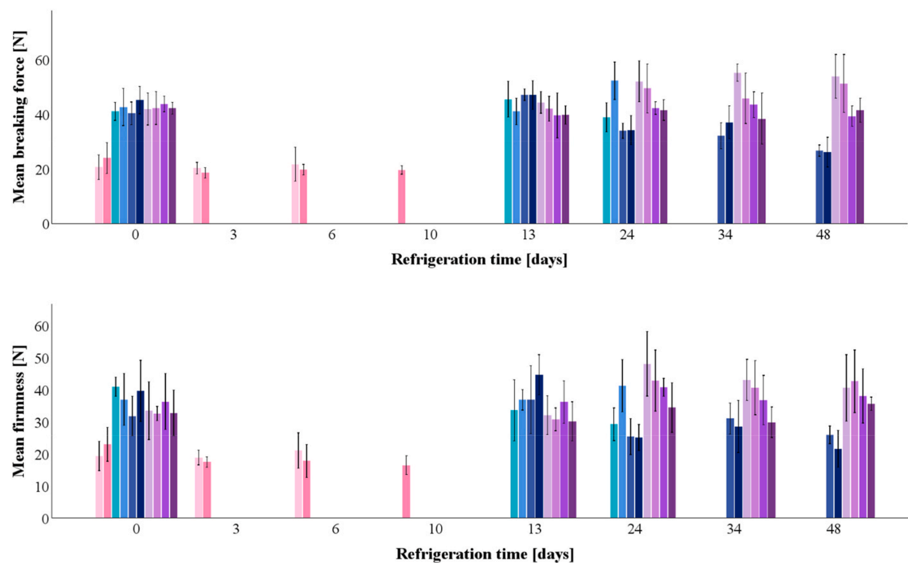

Fig. 4. (a) Mean breaking force (N) (GLM excluding control groups; SGS treatment: $p < 0.001$; pressure: p=<0.001; storage day: $p = 0.092$; packaging material: $p = 0.935$) and (b) Firmness at 60% compression (N) drip loss (%) of samples on through 48 days storage (GLM excluding control groups; SGS treatment: $p = 0.002$; pressure: $p = 0.006$; storage day: $p = 0.129$; packaging material: $p = 0.194$). The coloured bars represent groups C, CS, H400-L, H400–B, SH400-L, SH400–B, H600-L, H600–B, SH600-L and SH600–B.

aquatic species found a harder texture on samples subjected to HPP like cod (Christensen, Hovda, & Rode, 2017; Montiel, De Alba, Bravo, Gaya, & Medina, 2012; Rode & Rotabakk, 2021), salmon (Castrica et al., 2021; Lakshmanan, Miskin, & Piggott, 2005; Yagiz et al., 2009), yellowfish tuna (Tsai et al., 2022), haddock and herring (Karim et al., 2011). This phenomenon is likely related to protein denaturation and aggregation, changing the structural elements in the protein and its functional properties. According to Oliveira et al. (2017), myosin denatures at a pressure between 100 and 200 MPa, while actin at 200 MPa and sarcoplasmic proteins above 400 MPa. At pressures >300 MPa, proteins with a higher molecular weight begin to aggregate and remain stable due to the formation of hydrogen and disulphide bonds between the myosin heavy chain and actin. In contrast to our findings, Liu et al. (2023) found that the combination of 100% CO_2 and HPP at 450 MPa on Chinese spiced beef gave a consistently harder texture through storage. Perez-Won et al. (2020) reported that Coho salmon subjected to 50, 70 and 100% CO_2 and HPP at 150 MPa did not influence hardness. These discrepancies may be attributed to several factors like product type, pH,

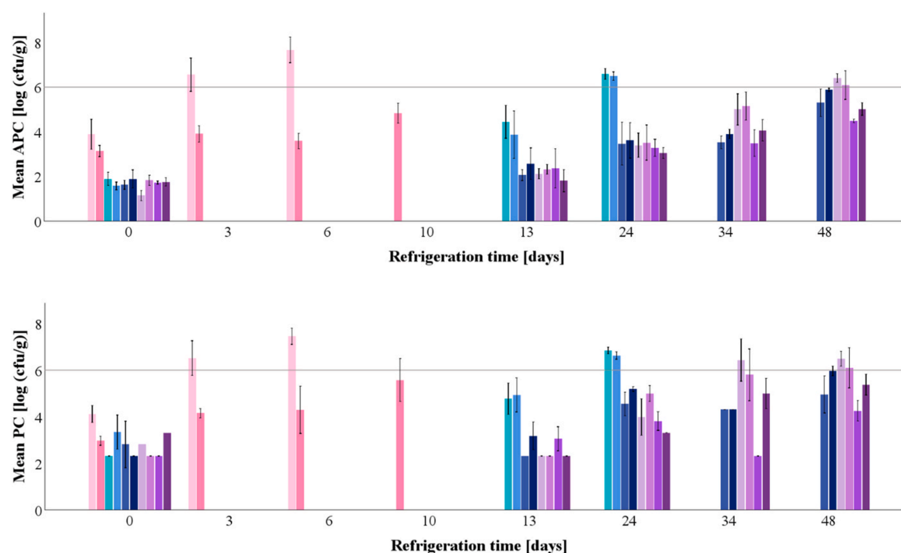

Fig. 5. The effect of various treatments on (a) total aerobic plate counts (APC) (GLM excluding control groups; SGS treatment: $p < 0.001$; pressure: $p < 0.001$; storage day: $p < 0.001$; packaging material: $p = 0.567$) and (b) total psychrotrophic plate counts (PC) (GLM excluding controls groups; SGS treatment: $p < 0.001$; pressure: $p = 0.118$; storage day: $p < 0.001$; packaging material: $p = 0.169$). The horizontal line at 6 log cfu/g represents the acceptable threshold before microbial spoilage. The coloured bars represent groups C, CS, H400-L, H400–B, SH400-L, SH400–B, H600-L, H600–B, SH600-L and SH600–B.

rigor mortis, proteolysis and processing conditions.

3.6. Microbial growth and pH

After processing on day 0, the microbial counts based on APC for those treated with HPP were all below quantification limits (2 log cfu/g). The control groups C and CS had a growth of 3.9 ± 0.39 and 4.1 ± 0.36 log cfu/g, respectively ($p < 0.001$). An aerobic count of >6 log cfu/g indicates spoilage (Dalgaard, 1995). Hence, the untreated control group was already spoiled after 3 days, with an APC and PC value of 6.6 ± 1.10 and 6.5 ± 1.0 log cfu/g, respectively. This was expected since rehydrated clip fish has a relatively short shelf life. The control group (CS) and samples treated with SGS and/or HPP significantly extended the microbiological shelf life as compared to the untreated control group (C). Only the control groups on day 0 had any HSPB growth, where groups C and CS had 2.0 ± 0.20 and 1.8 ± 0.24 log cfu/g, respectively ($p = 0.648$).

The APC was significantly affected by the SGS treatment ($p < 0.001$), pressure used ($p < 0.001$) and increased through storage ($p < 0.001$) (Fig. 5). There were no differences between the packaging materials used ($p = 0.567$). If an aerobic count of >6 log cfu/g is considered spoiled, the groups H400-L and H400–B have already surpassed the acceptable shelf li on day 24 at 6.6 ± 0.12 and 6.5 ± 0.10 log cfu/g, respectively, and hence excluded in further analyses. The PC was also significantly influenced by the SGS treatment ($p < 0.001$) and storage duration ($p < 0.001$). However, there were no differences between the pressure used ($p = 0.118$) and the packaging material ($p = 0.169$). The groups additionally treated with SGS (SH400-L, SH400–B, SH600-L, SH600–B) did not spoil after 49 days of storage, indicating the potential for a longer microbiological shelf life.

The groups SH600-L and SH600–B showed significantly lower microbial growth than those untreated with SGS (H600-L and H600–B). Still, the combination of SGS and 400 MPa (SH400-L and SH400–B) gave a significantly lower microbial growth than only applying a pressure of 600 MPa. This positively suggests the possibility of lowering the treatment pressure if SGS is included as a pre-treatment step before HPP. Introducing CO_2 provides antimicrobial properties. The SGS process also reduces the packaging material used when food is packaged in a modified atmosphere (Abel et al., 2019; Rotabakk, Birkeland, Jeksrud, & Sivertsvik, 2006; Sivertsvik & Birkeland, 2006). The present study is in line with Al-Nehlawi et al. (2014), who reported that HPP can enhance the effect of SGS on pork sausages by puncturing the cell membrane and helping with the easy dissolution of CO_2. There was a synergistic effect between CO_2 and HPP, where a lower pressure can also be applied with CO_2 to extend the microbial shelf life. Similar results were found in a recent study by Liu et al. (2023) on the assisted effect of CO_2 on HPP-treated Chinese spiced beef. In contrast, Dang et al. (2021) found that pre-cooked chicken breasts exposed to HPP at 600 MPa had a better microbial shelf life than those additionally treated with SGS. This was explained by the uneven absorption of CO_2 or different amounts of bacteria throughout the chicken fillet.

The measured pH from the Stomacher solution for all treated groups is presented in Table 3 pH was not influenced by the packaging material ($p = 0.673$) or pressure level ($p = 0.621$). pH significantly dropped throughout storage days, especially from day 0–13 ($p < 0.001$). On day 0, the samples treated with SGS have a slightly lower pH than those untreated ($p = 0.004$). The pH of the control groups was 6.7 ± 0.10 (group C) and 6.5 ± 0.05 (group CS) and remained relatively stable until the last sampling day. In addition, pH was negatively correlated to both APC ($r = -0.535$) and PC ($r = -0.462$). The lower pH of the SGS treated samples could be associated with the acidic effect of the dissolved CO_2 into the product (Rode & Rotabakk, 2021; Sivertsvik, Jeksrud, & Rosnes, 2002). Rode and Hovda (2016) observed that pH in HPP treated cod at 200 MPa and 500 MPa increased to day 7 before decreasing through storage. This was not observed in this study since the changes in pH were minimal.

4. Conclusion

There were minimal differences between the quality parameters analyzed on the packaging materials. Therefore, the bio-based food packaging used in this study can be an excellent environmentally friendly alternative to replace the conventional multi-material packaging and undergo mild treatment processes like SGS and HPP. The HPP treatment and the combination of SGS and HPP significantly contributed to the extended microbiological shelf life of the rehydrated clip fish. In addition, using HPP at a lower pressure with SGS gave a longer shelf life than using HPP alone at a higher pressure. The amount of FAA associated with flavour increased through storage time and SGS treatment. This could be related to protein oxidation and could be included as a parameter in future studies. In addition, using HPP may influence lipid oxidation. Hence, the assessment of lipid damage and a sensory panel can be considered to give a more accurate description of the organoleptic properties and sensory shelf life.

CRediT authorship contribution statement

Sherry Stephanie Chan: Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization. **Gøril Nygård Pettersen:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Tone Mari Rode:** Writing – review & editing, Methodology, Conceptualization. **Jørgen Lerfall:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Bjørn Tore Rotabakk:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

Table 3
Mean of pH measurement of all treatment groups (n = 6/group). "H" and "SH" denote processing with high-pressure or soluble gas stabilization and high-pressure processing, respectively. "400" and "600" represent high-pressure processing at 400 MPa or 600 MPa, respectively. "L" and "B" denote the PA/PE or BioPBS packaging materials, respectively.

pH Group	H400		SH400		H600		SH600		p-value[1]
Day/Package	L	B	L	B	L	B	L	B	
0	6.8 ± 0.03[b]	6.7 ± 0.02[ab]	6.7 ± 0.03[a]	6.7 ± 0.03[a]	6.7 ± 0.02[ab]	6.7 ± 0.02[ab]	6.7 ± 0.02[a]	6.6 ± 0.02[a]	$p_D < 0.001$*
13	6.5 ± 0.03	6.5 ± 0.04	6.5 ± 0.01	6.5 ± 0.01	6.5 ± 0.01	6.5 ± 0.02	6.5 ± 0.01	6.6 ± 0.02	$p_{pr} = 0.621$
24	6.4 ± 0.05	6.5 ± 0.04	6.4 ± 0.02	6.5 ± 0.03	6.5 ± 0.05	6.5 ± 0.03	6.5 ± 0.03	6.4 ± 0.03	$p_T = 0.020$*
34			6.4 ± 0.02[ab]	6.4 ± 0.04[ab]	6.5 ± 0.03[b]	6.4 ± 0.05[ab]	6.4 ± 0.05[a]	6.4 ± 0.04[ab]	$p_{pa} = 0.673$
48			6.4 ± 0.02	6.4 ± 0.03	6.4 ± 0.03	6.4 ± 0.02	6.4 ± 0.03	6.5 ± 0.03	

[1] p-values generated using general linear model (GLM) where p_D, p_{pr}, p_T, and p_{pa} represent storage day, pressure, SGS treatment and packaging.
* statistically significant when $p < 0.05$.
[ab] highlights significant differences ($p < 0.05$) pairwise between treatment groups on the same day.

the work reported in this paper.

Data availability

Data will be made available on request.

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