

Modified atmosphere packaging of flounder fillet: Modelling of package conditions and comparison of different flushing atmospheres for quality preservation

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Abstract

Modified atmosphere packaging (MAP) flushed in high CO₂ concentration with high gas barrier layer is often applied to preserve fish at chilled temperatures. Dissolution of CO₂ in fish product results in changes from the original CO₂ concentration and volume of packages. Maintenance of the desired CO₂ concentration under appropriate package volume is required for realising effectiveness in fish preservation and handling through distribution channel. In the present work, mathematical model for estimating package gas compositional changes along with volume decrease was developed by incorporating CO₂ dissolution dynamics into gas mass balance equation, and it was then validated by 200 g flounder packages flushed with atmospheres of 30 or 60% CO₂ and stored at 5°C. Then, the effect of different modified atmospheres (MAs) on preservation of the flounder fillets was examined for 100 g flounder packages stored at 10°C for 5 d. Package flushed with MA of CO₂ 60%/O₂ 30%/N₂ 10% was the best for preserving flounder fillet preservation, in terms of total aerobic bacteria inhibition and low volatile basic nitrogen content as major quality indexes.

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Introduction

Fresh fish is highly perishable because its high moisture, neutral pH, and high nutritional content favour bacterial growth and spoilage. Spoilage of fresh fish may also be caused by oxidation of lipid that may result in off-flavour development even only at slight oxidation reaction. In order to reduce the rate of deterioration, fresh fish products are stored and distributed under low temperature conditions. Modified atmosphere packaging (MAP) technology is often applied as an augmenting means to keep the quality of fish effectively under chilled temperature. In MAP of fish products, packages are flushed with a proper gas mixture containing CO₂, N₂, and O₂ for inhibiting microbial growth and oxidative reactions. CO₂ is essential element in the MAP as antimicrobial effect. N₂ is used as an inert gas to prevent package collapse. O₂ inhibits the growth of anaerobic bacteria and the colour degradation of red fish meat, but can

promote lipid oxidation and the growth of aerobic bacteria.

For successful application of MAP technology in seafoods, it is important to select the optimal CO₂/O₂/N₂ gas ratio specifically for the product. In a study on Atlantic cod fillets, Kuuliala *et al.* (2018) reported that 60% CO₂/5% O₂ concentration could reduce the microbial growth of aerobic bacteria, lactic acid bacteria, and *Pseudomonas* spp. at 4°C. Villemure *et al.* (1986) reported that cod fillets stored in 25% CO₂ and 75% N₂ had storage life twice as long as that of air-stored products at 0°C. For rohu fillet, MAP of 50% CO₂/50% O₂ was effective in extending shelf life from 11 d of vacuum pack to 16 d at 4°C, based on criterion of 7 log CFU/g in total bacterial count (Das *et al.*, 2021). Goulas and Kontominas (2007) reported that MAP of 70% CO₂/30% N₂ was the most effective in slowing down the spoilage of chub mackerel fillets at 2°C. Masniyom *et al.* (2002) reported that packaging seabass slices in 80 - 100%

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CO₂ atmosphere could extend the shelf life to more than 20 d at 4°C, significantly longer than 9 d of air package. Much more information still needs to be obtained for commodities even though some data are available for MAP effect on some products.

There are several variables in applying MAP to fresh seafood products: package atmospheric composition and ratio of gas to product (G/P ratio) (Randell *et al.*, 1995; Lee, 2021). Even though high CO₂ concentration, mostly in 20 - 100% range, works as a major component in package atmosphere, O₂ is often included to avoid concerns on toxin production by *Clostridium botulinum*, a pathogenic anaerobic bacterial species, and suppress reduction of trimethylamine oxide (TMAO) to trimethylamine (TMA). Particularly, lean fish relatively stable against oxidative degradation is suggested to be packed with MA containing O₂. Different modified atmospheres (MAs) need to be examined in their effectiveness of quality preservation, which may be different with different commodities. Since CO₂ is highly soluble in fish muscle consisting of high fat and water, the initially flushed gas mixture changes in its CO₂ concentration, thus altering package volume and antimicrobial effect. The resultant CO₂ concentration and volume are affected by the CO₂ solubility of product and G/P ratio. Extreme condition of high CO₂ concentration and small G/P ratio may cause collapse of package, which is undesired visually, and may create physical stress onto the fish meat. Appropriate G/P ratio above certain level is desired to be applied at the gas flushing operation for keeping the required CO₂ concentration throughout the storage, while too high G/P ratio occupies large space and costs high transportation expenses. Extremely low G/P ratio should be avoided for high-CO₂ MAP to prevent the package collapse. In most studies, G/P ratio varies in 0.4 - 5.0. Equilibrated CO₂ concentration and package volume would be determined through thermodynamic relationship of CO₂ dissolution onto the fish product, where mathematical modelling may be helpful for estimating possible outcome in the storage.

In the present work, the mathematical model for estimating volume and CO₂ concentration of high CO₂-MAP of fish was developed, and different package atmospheres were compared in the effect of product quality preservation. The flounder fillets were used as a typical fresh white muscle product for the study due to their major position in Korean consumption of fresh sashimi.

Materials and methods

Flounder fillets

Flounder fillets were purchased directly from a local store where live fish were subjected to scaling, heading, tailing, gutting, skinning, and washing on the day of the experiment. The prepared fillets were moved to the laboratory under chilled temperature conditions within 30 min.

Mathematical model to estimate CO₂ concentration and volume of flexible MAP of flounder fillets

A mathematical model to predict CO₂ pressure and void volume in CO₂-containing MAP of high gas barrier was established using mass balance and solubility CO₂ on the package using Eq. 1:

$$\frac{P_{CO_2,i}V_{h,i}}{R_gT} = \frac{P_{CO_2}V_h}{R_gT} + H_{CO_2}WP_{CO_2} \quad (\text{Eq. 1})$$

where, $P_{CO_2,i}$ = initial partial pressure of CO₂ (atm), $V_{h,i}$ = initial void volume (mL), V_h = void volume (mL) at equilibrium, R_g = gas constant (82.06 mL atm mol⁻¹ K⁻¹), T = temperature (K), H_{CO_2} = Henry's law constant of CO₂ solubility (mol kg⁻¹ atm⁻¹), W = product weight (kg), and P_{CO_2} = partial pressure of CO₂ at equilibrium (atm).

Henry's law constant, H_{CO_2} required in Eq. (1) was measured at different temperatures of 0, 5, 10, and 15°C by the constant volume method according to Wang *et al.* (2017) with some modification. Briefly, 250 g of fillets was put into 1 L glass jar sealed using a screwed metal lid with a silicon sampling port on it. Then, 200 mL of pure CO₂ (Union Gas Co., Gimhae, Korea) was injected through the silicon sampling port using an airtight syringe. The decreased CO₂ concentration at equilibration was analysed by gas chromatography (Varian CP 3800, Palo Alto, CA) for 1 mL of gas taken through the silicon sampling port. Alltech CTR I column (Alltech Associates Inc., Deerfield, IL) was used for separation of gases, and amount of gas was quantified with a thermal conductivity detector in the gas chromatography. He was used as the carrier gas. From the decrease in CO₂ concentration, the CO₂ absorbed by the flounder fillet (m_{ab}) was calculated from the mass balance. H_{CO_2} in unit of mol kg⁻¹ atm⁻¹ was obtained from the ratio of concentration of CO₂ absorbed by the flounder fillet (m_{ab}/W , mol/kg) to CO₂ partial pressure in the headspace using Eq. 2:

$$H_{CO_2} = \frac{m_{ab}/W}{P_{CO_2}} \quad (\text{Eq. 2})$$

Flexible package under normal atmospheric pressure is to have equilibration of total pressure with outside normal atmosphere of 1 atm, which can be stated as shown in Eq. 3:

$$P_{CO_2} + P_{N_2/O_2} = 1 \quad (\text{Eq. 3})$$

where, P_{N_2/O_2} = partial pressure sum of N_2 and O_2 which are negligible in their solubility in food product. Eq. 3 assumed that partial pressures of N_2 and O_2 were adjusted corresponding to the volume change.

In the flexible barrier packaging staying under normal atmospheric pressure, stable partial pressure of CO_2 and packaging void volume were obtained through repeated calculation using Eq. 1 under enforcement of Eq. 3. For two unknowns of the partial pressure of CO_2 and packaging void volume at equilibrium, the partial pressure of CO_2 was obtained from the initial void volume. Through a repeated process of obtaining the void volume from the partial pressure of CO_2 under the condition of Eq. 3 through the solution process, the stable partial pressure of CO_2 and packaging void volume were obtained, thus keeping package pressure same as external atmospheric pressure (1 atm).

For validating the above mathematical model, packages of 200 g flounder fillets were prepared in conditions different in CO_2 concentrations (50 and 70%) and void volumes (170 - 390 mL). 200 g of the fillets in a PET tray (105 × 135 × 30 mm; Yuna Pack, Busan, Korea) was placed in a multi-layer barrier film pouch of 150 × 180 mm (90 μm thickness; PP/PE/nylon/EVOH/nylon/PE/LLDPE; Sealed Air, Charlotte, USA). The packages were stored at 5°C for 24 h, and measured in CO_2 concentration and package volume, which were observed to reach equilibration without microbial influence from the preliminary experiment. The measured data were compared to the estimations. Package void volume was obtained from subtracting fish fillet volume and plastic material volume from the measured total package volume. Density, 0.97 g/mL of fish fillets estimated from moisture content of 72.5%, was applied to its volume. CO_2 concentration was measured by the gas chromatography for the 1 mL of gas sample taken out from the pouch headspace through a silicon sampling port as mentioned earlier. The total package volume

was measured by immersing the package in a graduated cylinder containing water at 5°C of the storage temperatures. To evaluate the quality of the model estimation, the root-mean-square of errors (RMSE) was calculated using Eq. 4:

$$RMSE = \sqrt{\frac{1}{n} \cdot \sum_{i=1}^n (E_i - O_i)^2} \quad (\text{Eq. 4})$$

where, E_i and O_i = the estimation and the observation values of CO_2 concentration or void volume, respectively, while n = number of predictions or observations.

Packaging treatments compared in quality preservation effect

Five different flushing atmospheres, including air as control, were compared for the package unit of 100 g fillets in their quality preservation effect at 10°C. As described earlier, the gas compositions of the treatments were selected to see the effect of O_2 and CO_2 on quality preservation. CO_2 concentration was varied to see the microbial growth inhibition. Concentration of O_2 often applied to prevent anaerobic headspace leading to possible botulinum risk was varied to see colour and oxidative quality changes. N_2 was used as a balance gas. The tried MAs with G/P ratio of about 2.9 were $CO_2(60):O_2(30):N_2(10)$, $CO_2(60):O_2(0):N_2(40)$, $CO_2(30):O_2(30):N_2(40)$, and $CO_2(30):O_2(0):N_2(70)$, where numbers in bracket are CO_2 , O_2 , and N_2 percentages of flushing gas mixture. The prepared fillets placed in a PET tray (90 × 135 × 18 mm) were packaged in pouches of a multi-layer gas barrier film (140 × 180 mm; 90 μm thickness; PP/PE/nylon/EVOH/nylon/PE/LLDPE) with flushing of different gas mixtures. While mathematical model was developed at 5°C with 200 g of flounder fillet, the storage test to see the MAP effect on quality preservation was conducted at 10°C with 100 g of flounder fillet, which could confirm the applicability of the developed model at different conditions of chilled temperature and package unit. Using smaller package unit of 100 g could also save the amount of sample preparation and room for sample storage without impairing the purpose and accuracy of the test. Slightly higher temperature of 10°C was expected to show more pronounced quality changes earlier.

Through the storage at 10°C for 5 d, packages were taken out in triplicate for each treatment and

measured in their headspace composition by a gas chromatography as described earlier. Then, the packages were opened to take out product samples for measuring quality attributes. The pH of fish fillet was measured using a pH meter (Orion Star A211, Thermofisher Scientific Inc., Fortcollins, USA) equipped with an electrode for surface contact measurement (927005MD, Thermofisher Scientific Inc.). For the total aerobic bacteria, 10 g of the fillet sample was collected in a sterile bag, 90 mL of 0.05% sterile peptone water was added, and then homogenised at 300 rpm for 4 min using a stomacher (Stomacher 400 circulator, Seward Limited, UK). The sample stock solution was diluted in 10-fold steps, and cultured on plate count agar (PCA, Difco Laboratories, Detroit, USA) at 30°C for 1 - 2 d to count colony forming units (log CFU/g). Total volatile basic nitrogen (TVB-N) was measured by the Conway micro-diffusion method. Sample of fish fillet (10 g) was homogenised with 40 mL of a 4% TCA solution for 10 s. The solution was filtered with Whatman No. 41 filter paper, and left at room temperature for 30 min. Next, 1 mL of indicator solution (0.01 g of bromocresol green + 0.02 g of methyl red + 10 mL of ethanol) was dispensed in the inner ring of the Conway unit, and then 1 mL of sample extract and 1 mL of saturated K₂CO₃ solution were dispensed in the outer ring. The cover was closed, fixed with a clip, mixed well, and reacted at 37°C for 60 min. The mixture was titrated with 0.02 N HCl solution, and TVB-N in mg/100 g was obtained using Eq. 5:

$$\text{TVB} - \text{N} = (A - B) \times (N_{\text{HCl}} \times A_N) \times \frac{[(W \times M / 100) + V_E] \times 100}{W} \quad (\text{Eq. 5})$$

where, A = titration volume of 0.02 N HCl (mL), B = titration volume of blank sample (mL), N_{HCl} = concentration of HCl, A_N = nitrogen atomic weight (14 g/mol), W = sample weight (g), M = moisture content of sample measured by drying at 105°C (%), and V_E = volume of trichloroacetic acid solution (mL).

Results and discussion

Estimation of package CO₂ concentration and volume

Figure 1 shows the CO₂ solubility on flounder fillets given in Henry's law constant (H_{CO_2}) as function of temperature to be used in estimating changes in package atmosphere and volume. The Henry's law constant was in the range of 0.043 - 0.093 mol kg⁻¹ atm⁻¹ at experimental temperatures of 0, 5, 10, and 15°C, which agreed with 0.042 - 0.055 mol kg⁻¹ atm⁻¹ of cod fillet at 0 - 4°C (Sivertsvik *et al.*, 2004). The CO₂ solubility decreased with temperature as found with moist or fatty foods such as fish, meat, and cheese (Jakobsen *et al.*, 2009; Chaix *et al.*, 2014). The temperature dependence of H_{CO_2} could be described using Eq. 6:

$$H_{\text{CO}_2} = 0.0001 T_c^2 - 0.0049 T_c + 0.0929 \quad (\text{Eq. 6})$$

where, T_c = temperature in degree Celsius (°C).

CO₂ solubility given by Eq. 6 as function of temperature could estimate the CO₂ partial pressure and volume of CO₂-flushed packages at different temperatures when substituted into Eq. 1. Solution of Eqs. 1 and 3 for different CO₂-flushing package conditions with applying Eq. 6 at 5°C could predict their partial CO₂ pressure and void volume to agree well with observed experimental values (Figure 2).

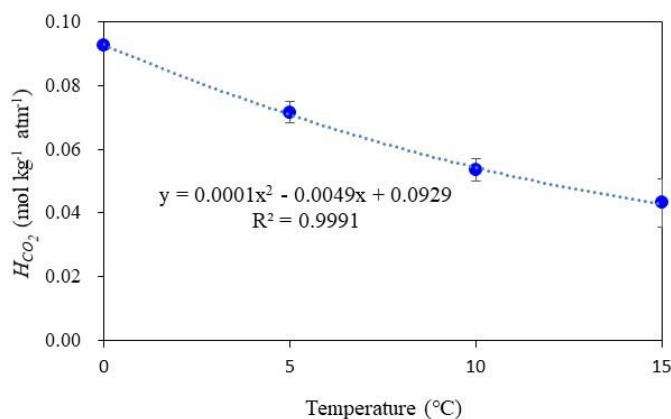


Figure 1. CO₂ solubility (H_{CO_2}) of flounder fillet as function of temperature. Vertical bars represent standard deviations.

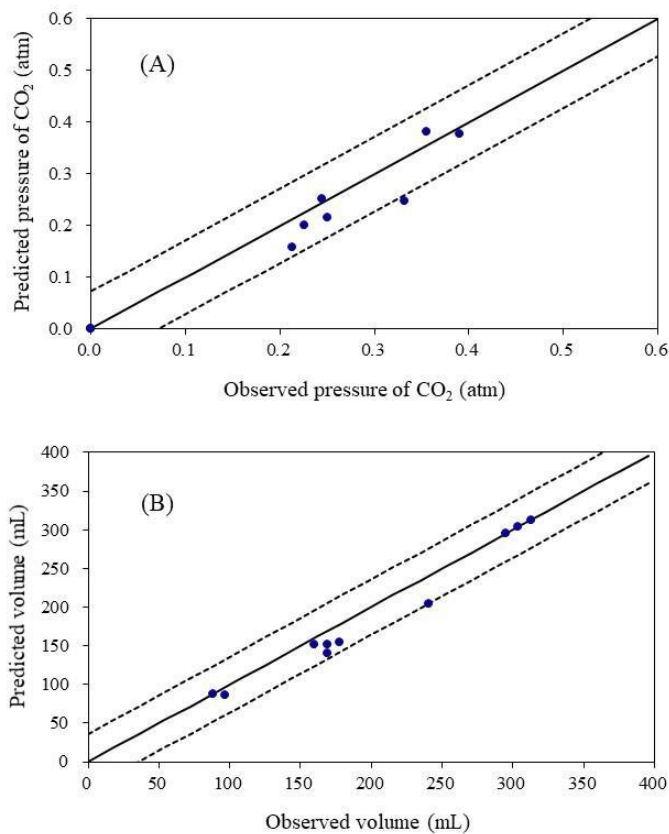


Figure 2. Comparison between the observed and predicted values of (A) the partial pressure of CO₂, and (B) void volume of the flounder fillet packages at 5°C. Dashed lines represent 95% prediction interval given as $\pm 2RMSE$ from the complete agreement.

This developed model would be useful to estimate the changes of package's CO₂ concentration and void volume at any expected temperature in range of 0 - 15°C for a given MAP condition of flushing gas mixture and G/P ratio, which may in turn help to find optimal MA condition at packaging operation.

Effect of different MA conditions on flounder fillet quality changes

Now that package atmospheric estimation model has been validated, packages of different MAs in 100 g unit were compared in the product quality preservation at 10°C storage. Decreases in package void volume of CO₂(60):O₂(30):N₂(10), CO₂(60):O₂(0):N₂(40), CO₂(30):O₂(30):N₂(40), and CO₂(30):O₂(0):N₂(70) packages were observed in 1-d storage at 10°C to be 81, 69, 30, and 30 mL, respectively. The observed volume decreases were slightly lower than the estimations for CO₂(60):O₂(0):N₂(40), CO₂(30):O₂(30):N₂(40), and CO₂(30):O₂(0):N₂(70) packages (estimates of 79, 43, and 45 mL, respectively), while there was good

agreement between observation and estimation for CO₂(60):O₂(30):N₂(10) package (81 vs. 80 mL). Variation in flushed package volumes having occurred in package production was thought to be one of reasons for the deviations. Figure 3 shows the change in package atmosphere through the 5-d storage at 10°C. Packages of CO₂(60):O₂(30):N₂(10) and CO₂(60):O₂(0):N₂(40) had about 48 and 43% CO₂ concentrations, respectively, through the storage after 1 d, which decreased from initial 60%. Packages of CO₂(30):O₂(30):N₂(40) and CO₂(30):O₂(0):N₂(70) showed less decrease from initial CO₂ concentration of 30% (24 and 20% after 1 d, respectively). These decreases in CO₂ concentration along with volume decrease were the results of CO₂ dissolution into the fish fillet. The observed CO₂ concentrations were close to the model prediction from Eqs. 1, 3, and 6 (50 and 22 - 23% for packages flushed MAs of 60 and 30%, respectively), except package of CO₂(60):O₂(0):N₂(40). Variation in sample and gas flushing operations resulting in inconsistent G/P ratio and initial package atmosphere might have caused the deviation from the prediction. Simplification of CO₂ dissolution based on Henry's law and Eq. 6 may also be an element contributing to the error. It is noted that increments of volume and CO₂ concentration are greater with flushing MA of higher CO₂ concentration, which should be considered in designing MAP of fish products to be stored at chilled temperatures.

Increase in CO₂ concentration and decrease in O₂ concentration reaching depletion in control package of air at day 3 could have been due to the respiration of aerobic microorganisms on the flounder fillet (Figures 3 and 4). In other packages of MAs containing O₂ (30%) and CO₂ (30 or 60%), decrease in O₂ concentration and increase in CO₂ concentration with storage were also observed: the changes were higher with the lower CO₂-concentration package of CO₂(30):O₂(30):N₂(40). For anoxic CO₂-flushed packages (CO₂(60):O₂(0):N₂(30) and CO₂(30):O₂(0):N₂(70)), the CO₂ concentration increase was milder than that of the O₂-containing MA packages probably due to O₂ absence effect on aerobic bacterial growth.

Aerobic bacterial growth on the flounder fillets in Figure 4 would have been affected by package atmosphere, and would in turn have influenced its change as discussed earlier: O₂ promotes the growth of aerobic bacteria while high CO₂ concentration suppresses their growth. The highest level in aerobic

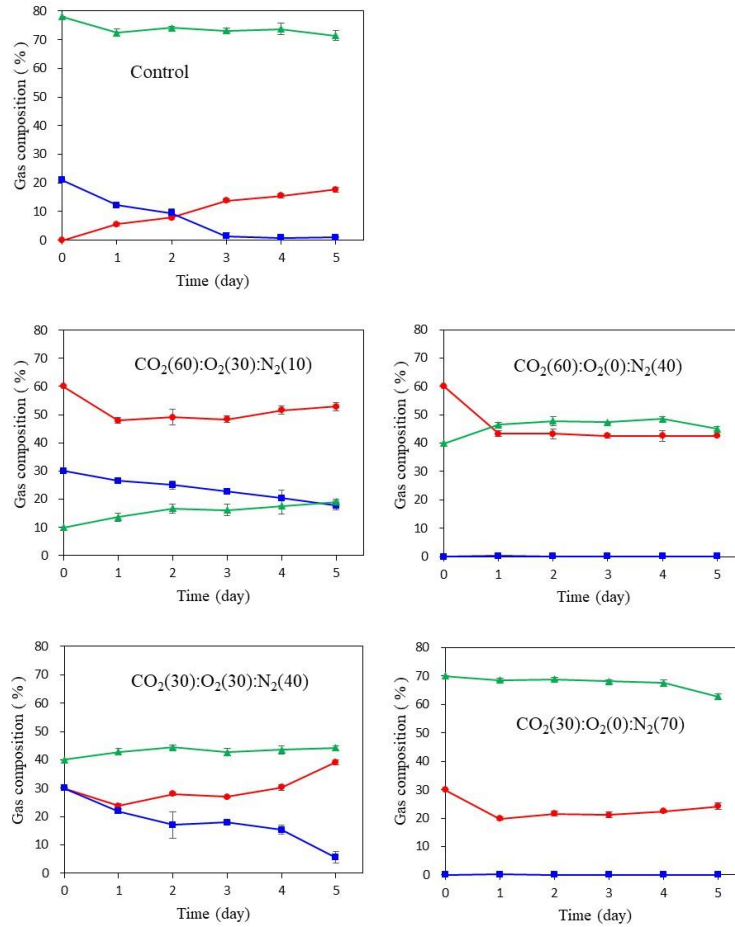


Figure 3. Gas concentration of flounder fillet packages flushed with different atmospheres, and stored at 10°C for five days. (●): CO₂; (■): O₂; and (▲): N₂. Vertical bars represent standard deviations.

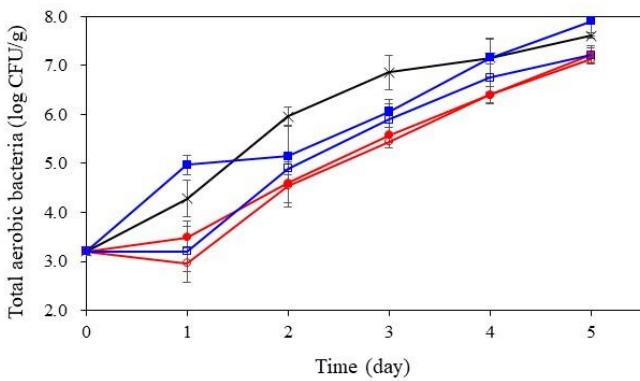


Figure 4. Total aerobic bacteria of flounder fillet packaged in different packaging treatments, and stored at 10°C for five days. (×): Control; (●): CO₂(60):O₂(30):N₂(10); (○): CO₂(60):O₂(0):N₂(40); (■): CO₂(30):O₂(30):N₂(40); and (□): CO₂(30):O₂(0):N₂(70). Vertical bars represent standard deviations.

bacterial count was maintained for control air package among the treatments from initial 3.20 to 7.61 log CFU/g at day 5 due to the initial 21% O₂,

which decreased its O₂ concentration to 12.2% and increased CO₂ concentration to 5.5% at day 5 (Figure 3). MA packages of high CO₂ concentrations (CO₂(60):O₂(30):N₂(10) and CO₂(60):O₂(0):N₂(40)) had the inhibited bacterial growth from 3.20 to 7.12 - 7.21 log CFU/g during the 5-d storage due to their high CO₂ concentration. Extension of lag time was obvious with these treatments. On the other hand, suppression of total aerobic bacterial growth was weak in 30% CO₂ MA packages (CO₂(30):O₂(30):N₂(40) and CO₂(30):O₂(0):N₂(70)), often applied commercially. The anaerobic package of CO₂(30):O₂(0):N₂(70) had only delayed the onset of bacterial growth followed by growth rate similar to that of control. Aerobic bacterial count was maintained less than 7.0 log CFU/g of commonly used microbial shelf life limit for 3-d storage for the two treatments (control and CO₂(30):O₂(30):N₂(40)), while more than 4 d were required to reach this limit for the other three treatments (CO₂(60):O₂(30):N₂(10), CO₂(60):O₂(0):N₂(40), and CO₂(30):O₂(0):N₂(70)). Inclusion of O₂ in 60% CO₂

MA package was found not to give negative impact on the fish preservation in terms of aerobic bacterial count.

The pH of flounder fillet decreased initially after flushing packages (Figure 5). The decrease was higher with higher CO₂ concentration of the flushing package atmosphere. This initial pH decrease could have come from lactic acid production of fish muscle carbohydrate catabolism and CO₂ dissolution in the muscle (Hamada-Sato *et al.*, 2005; Lee, 2021). The absence of CO₂ in control treatment would have resulted in the highest pH among the treatments. Then, a tendency of slight pH increase after 3 d with the most significant degree for control treatment was observed. This later pH increase was presumed to have been caused by nucleotide metabolism and aerobic bacterial growth producing basic compounds, which was also confirmed by the highest pH increase in control treatment. The similar pH changes were also observed for slices of seabass and rohu fish packaged with high CO₂ MA, and stored at chilled temperature (Masniyom *et al.*, 2002; Das *et al.*, 2021). Even though there appeared to be a large variation among sample measurements, the tendency of pH change in Figure 5 agreed with usual storage of fresh fish products occurring along with TBV-N increase (Hamada-Sato *et al.*, 2005; Lee, 2021).

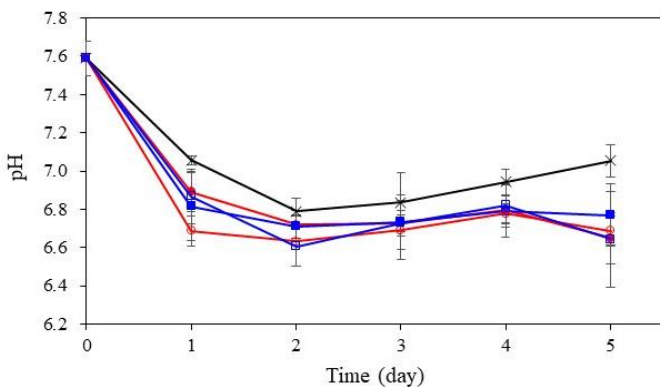


Figure 5. Change in pH of flounder fillet packaged in different packaging treatments, and stored at 10°C for five days. (×): Control; (●): CO₂(60):O₂(30):N₂(10); (○): CO₂(60):O₂(0):N₂(40); (■): CO₂(30):O₂(30):N₂(40), and (□): CO₂(30):O₂(0):N₂(70). Vertical bars represent standard deviations.

TVB-N content of flounder fillets remained low with only slight increase for all the packaging treatments until day 2 (Figure 6). From day 4, control

and CO₂(30):O₂(0):N₂(70) showed sharp increase in TVB-N value reaching 25.5 mg/100 g. Packages of CO₂(60):O₂(30):N₂(10) and CO₂(30):O₂(30):N₂(40) could keep low level of TVB-N below 20 mg/100 g for 5 d, which is often used as a critical limit of freshness in shelf life determination of fresh fish products. Even though the package of CO₂(60):O₂(0):N₂(40) maintained low TBV-N level until day 4, drastic increase was observed on day 5. This high increase for the high CO₂ package treatment having inhibited microbial growth (Figure 4) was surprising considering that TBV-N increase is often caused by microbial growth. As a possible explanation for low TVB-N level of CO₂(60):O₂(30):N₂(10), the included O₂ would have inhibited reduction of TMAO to TMA. Since TMA constitutes a significant part of TVB-N (Bekhit *et al.*, 2021), the inhibited TMAO reduction could help keep the low TVB-N content. Similar reasoning may explain the consistently low TVB-N observed for CO₂(30):O₂(30):N₂(40) treatment. Das *et al.* (2021) also reported that package of 50% CO₂/50% O₂ could maintain low TVB-N values in rohu fish stored at 4°C. Considering TVB-N as a major index for product shelf life, CO₂(60):O₂(30):N₂(10) could be the best packaging condition for flounder fillet preservation. This package treatment is also desirable in the perspective of microbial quality preservation. When compared with common commercial MAP condition of CO₂(30):O₂(30):N₂(40), the treatment of CO₂(60):O₂(30):N₂(10) could extend shelf life at least by 1 d based on aerobic bacterial count or TVB-N.

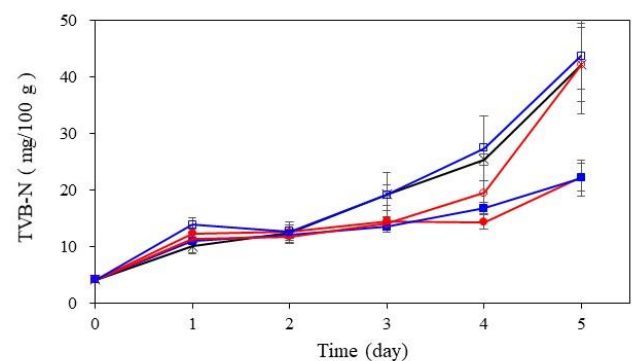


Figure 6. Total volatile basic nitrogen (TVB-N) of flounder fillet packaged in different packaging treatments, and stored at 10°C for five days. (×): Control; (●): CO₂(60):O₂(30):N₂(10); (○): CO₂(60):O₂(0):N₂(40); (■): CO₂(30):O₂(30):N₂(40); and (□): CO₂(30):O₂(0):N₂(70). Vertical bars represent standard deviations.

The effect of MAP condition on quality preservation in the present work could be the result for white flesh fillets. The effect is expected to be different when applied to red fish fillets being different from white muscle in composition particularly in myoglobin pigment content (Lee, 2021). Future work is to study the effect of different flushing MA gas mixtures on the quality of red muscle fish product.

Conclusion

Incorporating CO₂ dissolution into mass balance of CO₂ on the barrier flexible package of MA could estimate the changes in its volume and CO₂ concentration under expected packaging and temperature conditions. The model could help to find optimal MA condition at packaging operation for attaining the desired CO₂ concentration and volume in the storage. Flushing atmosphere of CO₂(60):O₂(30):N₂(10) was found to be the best packaging condition for flounder fillet preservation in terms of microbial and chemical quality deterioration. CO₂(60):O₂(30):N₂(10) was more effective in maintaining the quality of flounder fillets than control and commercial application of CO₂(30):O₂(30):N₂(40).

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