



# Independent and combined effects of high pressure, microwave, soluble gas stabilization, modified atmosphere and vacuum packaging on microbiological and physicochemical shelf life of precooked chicken breast slices

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## ABSTRACT

The study aimed at extending shelf life without compromising quality of precooked chicken breast slices by various packaging and processing technologies. The slices were packaged in modified atmosphere packaging (MAP, 40% CO<sub>2</sub>, 60% N<sub>2</sub>), vacuum packaging (VAC, 93% vacuum), processed with high pressure (HPP, 600 MPa, 2 min), microwave volumetric heating (MVH, 1 kW, 3 × 15 s), soluble gas stabilization (SGS, 100% CO<sub>2</sub>, 18 h, 1 °C) followed by MAP (SGS-MAP), SGS followed by VAC (SGS-VAC), SGS followed by HPP (SGS-HPP), and SGS followed by MVH (SGS-MVH). The eight treatments were studied for their microbial and physicochemical quality during 4 °C storage for maximum 119 d. The results showed that MAP, the most common commercial packaging of ready-to-eat chicken, was slightly superior to VAC but much inferior to HPP, MVH, and SGS in microbial control. HPP, with/without SGS, was the most efficient method extending the shelf life of precooked chicken to more than three months. SGS improved microbial inhibition compared to non-SGS, but synergetic effects with HPP or MVH on microbial and physicochemical quality were insignificant. All treated chickens shared relatively comparable color, texture, pH and drip loss. These quality attributes changed marginally during storage while lipid oxidation increased markedly. HPP and MVH reduced lipid oxidation right after treatment and during storage. SGS suppressed the lipid oxidation but this effect did not last longer than two weeks of storage.

## 1. Introduction

Poultry meat is the most consumed meat products worldwide, exceeding pork, beef and sheep meat (OECD., 2020). Its global consumption has been increasing from 12.3 kg/capita in 2008 to 14.2 kg/capita in 2018 and is forecasted to continue the increase in upcoming years (OECD., 2020). As poultry meat is highly perishable, its shelf life has to be extended to meet producer and consumer requirements (Lerasle et al., 2014). Pathogens, e.g. *Salmonella* spp. and *Campylobacter* spp. present a challenge for the poultry industry in most parts of the world and post-harvest processing is also one of the strategies to provide food safety. The recent increasing prevalence of thermoresistant *Campylobacter* spp. (Rossler et al., 2019) is renewing the relevance of studying the food safety impact of processing.

High pressure processing (HPP) is a non-thermal technology offering extended shelf life, enhancing chicken breast fillet freshness by reducing

volatile basic nitrogen, inhibiting pathogens (*Salmonella*, *Listeria*, and *Escherichia coli*) in chicken products (Chien et al., 2016; Kruk et al., 2011; Stratakos et al., 2015). Pressure higher than 300 MPa may have negative impact on flavor, odor, color and texture of chicken breast fillets (Del Olmo et al., 2010; Kruk et al., 2011). It is consistently found that HPP has accelerated lipid oxidation in chicken product and the critical level of pressure where lipid oxidation initiates is found at 450 or 500 MPa for 5 min (Bolumar et al., 2014; Kruk et al., 2011).

Microwave is a thermal technology that has increasingly gained popularity in food processing due to its high energy efficiency, reduced cooking time, ease of use and low maintenance (Chandrasekaran et al., 2013). Microwave heating is caused by interaction of electromagnetic radiation with dielectric materials. Unlike convection or conduction heating, microwave radiation penetrates directly into the material and generates heat throughout the volume of the material, therefore it is also called volumetric microwave heating (MVH) (Zhu et al., 2007).

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Microwave (frequency 2450 MHz) can be used to pasteurize poultry products, e.g. eliminating *Salmonella senftenberg* in turkey drumsticks in 120 s (Teotia and Miller, 1975), *Listeria monocytogenes* in chicken portions by 900 W for 60 s from initial inoculum of 6.2 log CFU/g (Zeinali et al., 2015) or in chicken breast by 1100 W for 120 s or 240 s from initial inoculum of 2.4 and 8.7 log CFU/g respectively (Morey et al., 2012), *E. coli* O157:H7 in chicken portions by 800 W for 35 s from initial inoculum of 6 log CFU/g (Apostolou et al., 2005). Cunningham (1980) suggested that consumers could extend the storage life of fresh meat bought from supermarkets by microwaving them for 15–20 s before placing them in the refrigerator. However, uneven surface heating is always the major concern in both domestic and experimental microwave (Fakhouri and Ramaswamy, 1993; Goksoy et al., 1999; Mullin and Bows, 1993). Microwave affects meat appearance (partial cooking) (Goksoy et al., 2000), increases the amount of malondialdehyde (MDA) and lipid oxidation fluorescent products in all chicken parts (breast, thigh, rib, drumstick and wing).

Modified atmosphere packaging (MAP) is well documented to provide inhibitory effects on microbial growth. MAP using 70% CO<sub>2</sub> and 30% N<sub>2</sub> gave 25-d and 21-d shelf life for chicken stored at 2 °C and 3 °C, respectively, and the higher concentration of CO<sub>2</sub> the longer shelf life (Sawaya et al., 1995). A method to maximize the effect of MAP is to dissolve CO<sub>2</sub> into the product prior to packaging, and it is called soluble gas stabilization (SGS) (Sivertsvik and Jensen, 2005). The solubility of CO<sub>2</sub> into the meat product is dependent on temperature, initial pressure of CO<sub>2</sub>, gas/product volume ratio, the water content and surface area of the products (Sivertsvik et al., 2004; Sivertsvik and Jensen, 2005). SGS can improve microbial shelf life without compromising physicochemical and sensorial quality as well as reduce package collapse and increase filling degree of chicken breast fillets (Rotabakk et al., 2006).

The effect of individual HPP, MVH, MAP, VAC and SGS treatments

on physicochemical and microbiological quality of non-cooked chicken during storage were well studied earlier (Kruk et al., 2011; Luckose et al., 2015; Morey et al., 2012; Rotabakk et al., 2006; Sawaya et al., 1995). However, the effect of these treatments on quality of precooked chicken during storage have received less attention, except for HPP on microbiological quality of precooked chicken (Patterson et al., 2010, 2011). Also, to our knowledge, the combined effects, e.g. SGS with HPP or MVH on the shelf life of precooked chicken breast slices have not been evaluated elsewhere.

The objective of this study was to investigate the efficiency of food packaging and processing technologies (modified atmosphere packaging, vacuum packaging, high pressure, microwave, and CO<sub>2</sub> soluble gas stabilization) in individual and combined manner, in order to increase the shelf life of precooked chicken slices while maintaining the quality.

## 2. Materials and methods

### 2.1. Materials

Fresh chicken breast fillets were obtained from Den Stolte Hane AS (Nærbø, Norway). The fillets were kept at 1 °C after receiving and processed within less than 2 h from slaughtering.

### 2.2. Experimental design

An outline of experimental design is presented in Fig. 1. In brief, all chicken breast fillets were cooked, cooled, then divided into two sets: non-soluble gas stabilization (non-SGS) and soluble gas stabilization (SGS) pretreatment. For non-SGS set, precooked and cooled chicken fillets were cut with a sharp knife into slices (~5 mm thick). Each slice

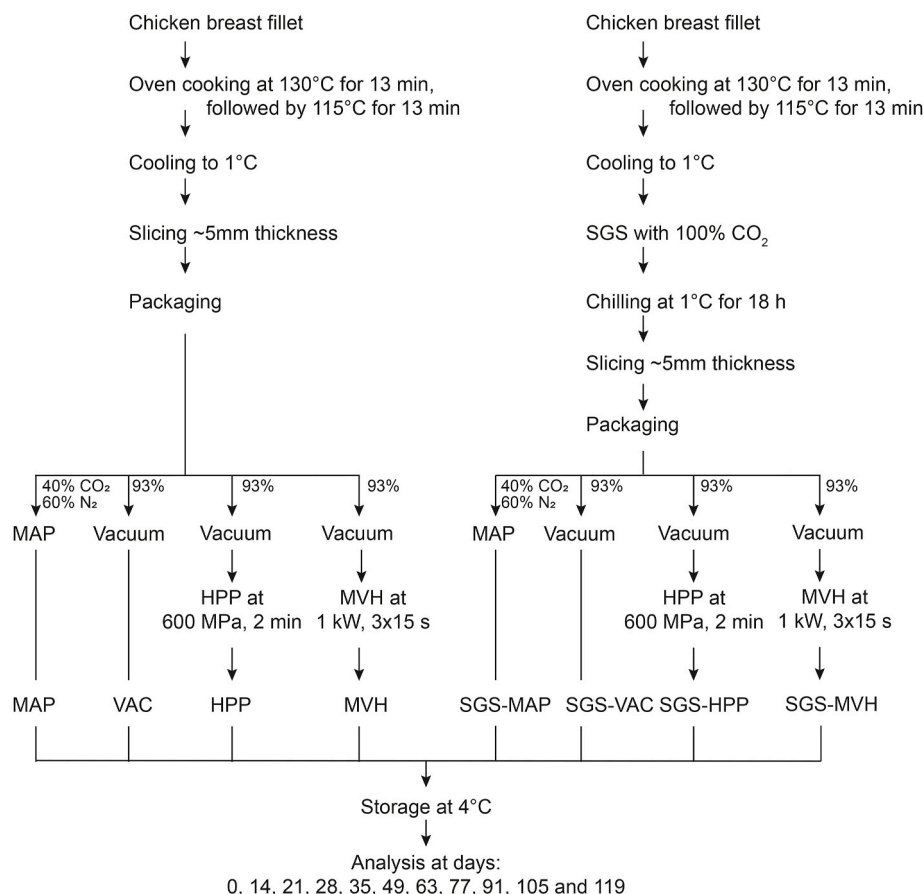


Fig. 1. Outline of experiment.

was packaged into either modified atmosphere packaging (MAP) bag or vacuum packaging (VAC) bag. All MAP bags and some VAC bags were stored in a cool room (4 °C) immediately after packaging. The remaining VAC bags were used to apply high pressure processing (HPP) and microwave volumetric heating (MVH) before storing in the cool room. For SGS set, the only difference with non-SGS set was that precooked and cooled chicken fillets were pretreated with SGS for 18 h prior to cutting. All subsequent steps were identical as non-SGS set.

### 2.3. Precooking

The fresh chicken breast fillets were removed from the skin and placed on racks (8 fillets/rack) for cooking in a convection oven (model Combi FCV/E10, Zanussi Professional, Pordenone, Italy). The cooking was conducted in two continuous thermal cycles: 130 °C for 13 min followed by 115 °C for 13 min. A profile of cooking parameters (temperature of oven and chicken, and relative humidity) was recorded (Supplementary material). Relative humidity inside the oven fluctuated from 10 to 55% during cooking. The core temperature of chicken fillets was ~90 °C at the end of cooking. After cooking, the chicken was quickly cooled to 1 °C by a freezer at -20 °C.

### 2.4. Modified atmosphere packaging (MAP)

Each chicken slice (~10 g) was packed into a 80- $\mu$ m standard vacuum/MAP plastic bag (Arne B. Corneliussen AS, Oslo, Norway) that can withstand heat up to 95 °C for 1 h. The atmosphere was evacuated and subsequently flushed with a gas mixture of 40% CO<sub>2</sub> and 60% N<sub>2</sub> into the bag. The CO<sub>2</sub>/N<sub>2</sub> ratio was selected based on the optimal MAP condition for extending the shelf life of roast chicken (Guo et al., 2018). MAP sliced chicken bags were stored at 4 °C until analysis. At each sampling day, the gas composition in the head space was measured using an oxygen and carbon dioxide analyzer (Checkmate 9900 analyzer, PBI-Dansensor, Ringsted, Denmark).

### 2.5. Vacuum packaging (VAC)

The slice was placed in a 80- $\mu$ m standard Sous-vide plastic bag (Arne B. Corneliussen AS, Oslo, Norway) that can withstand heat up to 120 °C for 1 h. The bag was vacuum packaged (93% vacuum) in a vacuum packer (model Webomatic SuperMax, Webomatic Maschinenfabrik GmbH, Bochum, Germany). Some sliced chicken bags were stored at 4 °C until analysis, and some bags were subjected to HPP and MVH processing.

### 2.6. Soluble gas stabilization (SGS)

SGS was performed according to the method of Rotabakk et al. (2006). Precooked and cooled chicken breast fillets were placed inside a stainless-steel tray (35 × 29 × 5 cm). The tray was placed inside a heat-sealed 20- $\mu$ m PA/70- $\mu$ m PE bag (700 × 500 mm, Star-pack produktie B.V., The Netherlands). The atmosphere in the bag was evacuated by a vacuum packer (model Webomatic SuperMax, Webomatic Maschinenfabrik GmbH, Bochum, Germany) and flushed with 100% food grade CO<sub>2</sub>. The initial atmosphere inside the bags immediately after packaging contained 97.1% CO<sub>2</sub>, 0.27% O<sub>2</sub> and 2.63% N<sub>2</sub>. The SGS-bags were sufficiently large to ensure excess availability of CO<sub>2</sub>. The chicken bags were stored at 1 °C for 18 h. The atmosphere inside the bags after 18-h chilling was 98.1 ± 0.8% CO<sub>2</sub>, 0.1 ± 0.1% O<sub>2</sub> and 1.8 ± 0.7% N<sub>2</sub>.

### 2.7. High pressure processing (HPP)

HPP was performed in a lab-scale high pressure unit QFP 2L-700 (Avure Technologies Inc., Columbus, USA). Distilled water was used as transmitted pressure medium in the vessel. Vacuum-packed samples

were pressurized at 600 MPa for 2 min. The temperature inside the vessel at holding time was recorded to maximum 39 °C. Come-up time was approximately 100 s for 600 MPa and decompression was immediate. The duration of treatment (2 min) did not include the come-up time. The 600-MPa/2-min condition was selected based on the previous findings (Patterson et al., 2010, 2011), which allowed the reduction in counts of *L. monocytogenes* and pressure resistant *Weissella viridescens* below detection limit (<1.7 and < 1 log CFU/g respectively). The condition was considered appropriate for the cooked poultry meat from both commercial and scientific viewpoint due to achievable pressure level, short hold time and minimized enzymatic changes (Patterson et al., 2010).

### 2.8. Microwave volumetric heating (MVH)

Vacuum-packed samples (100 g chicken in 10 individual vacuum-packed bags per batch of microwave operation) were heated in a laboratory microwave autoclave (Gigatherm AG, Flawil, Switzerland) operated at 2450 MHz, 1 kW (Rosnes and Skipnes, 2018), and 1.5 bar set pressure above atmosphere (2.5 bar accumulated inside the microwave cavity). The power was introduced in three cycles of 15 s with 10-s interval (45-s heating in total). The MVH condition was selected after performing several screening trials with two criteria taken into account, i.e. the temperature of chicken slices reached ~72 °C and avoidance of the packaging melting. After microwave heating, samples were immediately cooled in an ice box followed by another packaging (50% vacuum). The samples were stored in a refrigerator at 4 °C until analysis.

### 2.9. Texture

Texture analysis was performed using a Texture Analyzer XT Plus (Stable Micro Systems Ltd., UK) which equipped with 50-kgf cell load. The chicken slice was cut using the probe HDP/BSK blade set with a knife. The setting of test as follow: test mode compression, test speed 2 mm/s, trigger force 5 gf. As thickness and width of manual cutting chicken slices were not always consistent, the cutting force was normalized for thickness (4 mm) and width (25 mm) of slices. The cutting presented is defined as the maximum force at 4-mm distance of cutting through the sample (F<sub>4mm</sub>) multiplied by 25 (mm) and divided by the real width of slice (W, mm).

$$\text{Cutting force (kgf)} = \frac{F_{4\text{mm}} \times 25}{W}$$

### 2.10. Color

The color of chicken was measured using VeriVide's DigiEye system (VeriVide Ltd., Leicester, UK) equipped with a DSLR camera (Nikon D90, Japan). The camera captures an image of 4288 × 2848 pixels and a resolution of 96 dpi. Before capturing image, the camera was white balanced and calibrated with the color chart provided with the equipment. The L\*, a\*, and b\* components were recorded at a D65 standard illuminant. The captured image was analyzed for CIELAB color scale using the DigiEye 2.9 software.

### 2.11. Drip loss

Drip loss was measured on each chicken slice (~10 g) immediately after packaging/processing and during chilled storage as formula below:

$$\text{Drip loss (\%)} = \frac{\text{Meat weight at packaging} - \text{Meat weight at sampling day}}{\text{Meat weight at packaging}} \times 100$$

### 2.12. Microbiological analysis

On sampling days, chicken slices were taken from the refrigerated

storage and were quantified for total viable count (TVC), lactic acid bacteria (LAB), *E. coli*, *Listeria*, and *Campylobacter*. Chicken sample (~10 g) was placed in a sterile filter bag (Separator 400 Blender Bag, Grade Products Ltd., Leicestershire, UK) with 90 mL (diluted 1:10) of 1% sterile peptone water and mashed for 4 min using a stomacher (Smasher, BioMerieux Industry, MO, USA) at the fast speed setting. The filtered liquid was serially diluted with peptone water and spread onto plates of Plate Count Agar (Merck-Millipore Corp., Billerica, USA), de Man, Rogosa and Sharpe (MRS) agar (Merck-Millipore Corp., Billerica, USA), 3M Petrifilm *E. coli*/Coliform count plates (3M, MN, USA), CM1080 Brilliance *Listeria* agar base with SR0227 Brilliance *Listeria* Selective Supplement (Oxoid Ltd., Basingstoke, UK), CM0739 *Campylobacter* Blood-Free Selective Agar Base with SR0155 CCDA Selective Supplement (Oxoid Ltd., Basingstoke, UK). The plates were incubated at 30 °C (TVC, LAB, and *Listeria*) or 37 °C (*Campylobacter* and *E. coli*) for 48 h. Following incubation all colonies on plates were counted. The results were calculated and given as log CFU/g sample. For statistical analysis convenience, if all dilutions produced zero colony (below detection limit 1.3 log CFU/g, < 1 CFU per plate containing 0.5 mL of 1:10 solution), 0.5 would be substituted at the first dilution (Parshionikar et al., 2009).

### 2.13. pH

The mashed, filtered chicken liquid from microbiological analysis was used to measure the pH using a Mettler Toledo pH meter (Mettler Toledo, Columbus, Ohio, US) equipped with LE410 electrode.

### 2.14. Lipid oxidation

The extent of lipid oxidation in chicken samples was assessed by measuring thiobarbituric acid reactive substances (TBARS). TBARS was expressed as mg of malondialdehyde (MDA) per kg sample. Two grams of chicken sample were added to 4 mL of 2 N perchloric acid and 3 µL of BHT, homogenized at 9500 rpm for 5 min on the Ultra Turrax homogenizer (IKA T25, IKA®-Werke GmbH & Co. KG, Staufen, Germany), followed by filtering through Whatman No.1 filter paper. The filtrate was used to measure the extent of lipid oxidation (MDA/kg) using commercial Lipid Peroxidation (MDA) Assay Kit from Sigma Aldrich (Cat No. MAK085).

### 2.15. Data analysis

Means and standard deviations calculated from triplicate analysis of microbiology, pH and lipid oxidation, and from quintuplicate analysis of drip loss, color, texture were presented. Analyses of variance ( $p < 0.05$ ) and Tukey HSD post-hoc comparison were performed in SAS 9.4 (SAS Institute Inc., Cary, New York). For overall evaluation of data, probabilistic principal component analysis (PPCA) was performed using Matlab R2018a (The MathWorks Inc., Natick, MA). Pearson's correlation coefficients were calculated and plotted in R using Hmisc and Corplot packages.

## 3. Results and discussion

### 3.1. Texture

Texture is one of important consumer quality preferences towards meat products. Generally increased hardness in cooked muscle-based food during storage is unfavorable as this could affect consumer acceptability (Ganhao et al., 2010). Texture of meat products during storage can be influenced by several factors, e.g. dehydration (Candogan and Kolsarici, 2003), cooking method (Dai et al., 2014; Ganhao et al., 2010; Wills et al., 2006), oxidation reaction (Dai et al., 2014; Ganhao et al., 2010), and bacterial load (Jay, 1965). Storage of cooked products usually increase the hardness due to the first three to four factors, while storage of uncooked products usually decreases the hardness due to the

last two factors.

Effect of treatments and storage length on texture of precooked chicken slices is shown in Fig. 2. As seen, a non-significant increase in cutting force over the storage time was observed in all samples. The hardness increase in cooked chicken products during refrigerated storage was observed more obviously in previous studies (Prasad et al., 2011; Santos et al., 2019). Also, there were no statistically significant differences between treatments immediately after processing (day 0) and during storage. Exceptions were observed in MAP and SGS-MAP treated chicken which were significantly softer than VAC at day 14. The lower cutting force at this storage time was not due to the decline of texture during storage since there were no significant differences between day 14 and day 0 in MAP and SGS-MAP samples. In comparison between the hardness of MAP and that of VAC, the present results were in agreement with previous work (Bartkowski et al., 1982; Garcia-Esteban et al., 2004) that MAP protect the product from hardening better than VAC. The stability of texture between samples indicated that additional processing (HPP, MVH) did not cause any negative effect on texture of precooked chicken.

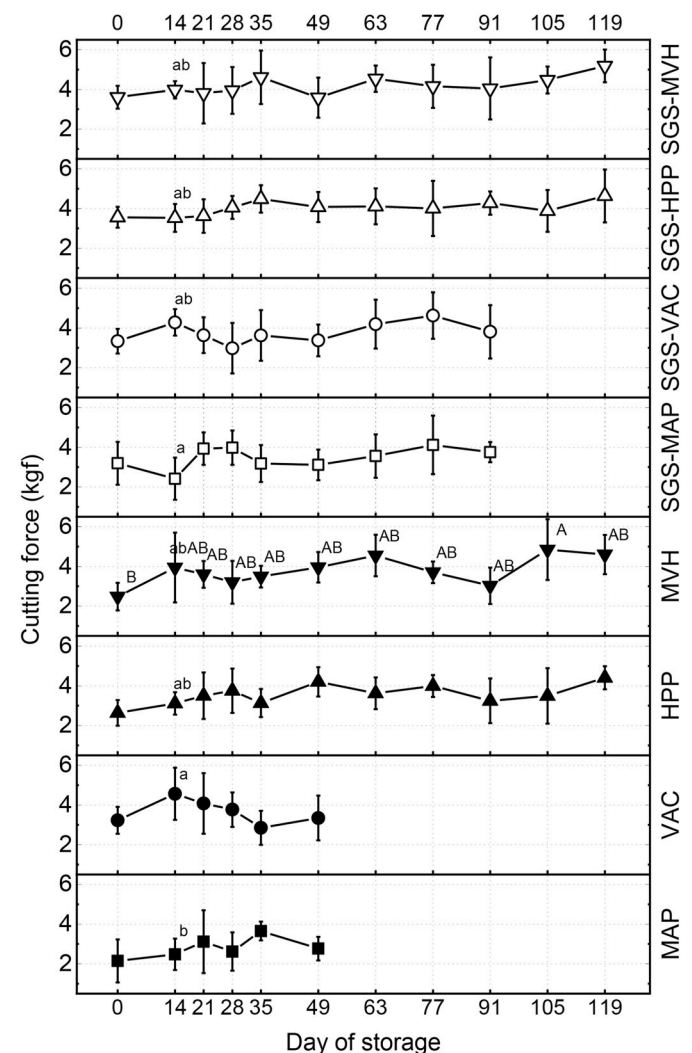


Fig. 2. The effect of storage duration and varying treatments on the texture of precooked chicken. Each point is a mean of 5 replicates. Small letters (a, b) indicate significant difference ( $p < 0.05$ ) between treatments at the same time point, and capital letters (A, B) indicate significant difference ( $p < 0.05$ ) between days of storage in a treatment. Error bars indicate standard deviations.



### 3.2. Color

Color is a major quality attribute of chicken products influencing the selection decision as well as final product satisfaction of consumers (Fletcher, 2002). Color stability over storage is one of the targets of chicken processing. In general, the pink or red appearance of cooked chicken meat is associated with undercooking and is particularly undesirable (Fletcher, 2002). Several factors influencing the color of cooked chicken products include biological variations, body parts of animal, sex, strain, muscle pH, processing procedures, chemical exposure, cooking temperature, irradiation, and heme pigments' reactions (Fletcher, 2002).

Changes in color of precooked chicken processed with varying treatments over chilled storage are presented in Fig. 3. Generally, most samples followed the same trends: lightness ( $L^*$ ) varied in the first 14–21 d then remained stable, redness ( $a^*$ ) slightly decreased over storage, and yellowness ( $b^*$ ) slightly increased over storage. The slight increase in yellowness observed in all samples during storage may be caused by storage-induced oxidation processes e.g. pigment oxidation, lipid oxidation (Wang et al., 1995). The decrease in redness and the increase in lightness could be attributed to the oxidation of denatured globin and the oxidative cleavage of hematin pigment which releases iron from heme molecule (Estevez and Cava, 2004).

Lightness of precooked chicken was sensitive to SGS as evidenced by the significantly lower  $L^*$  value of SGS samples compared to non-SGS samples at day 0. This phenomenon could be related to the high concentration of  $CO_2$  (100%) and the absolute absence of oxygen in the

chicken during SGS process. The darkening induced by high  $CO_2$  concentration in meat products was previously reported and interpreted based upon the primary formation of metmyoglobin (Arvanitoyannis and Stratakos, 2012; Ogilvy and Ayres, 1951). The  $CO_2$ -induced darkening was more noticeable in red meat than in white meat (Rao and Sachindra, 2002). After being stored at 4 °C for 14 d, the lightness of SGS samples significantly increased while that of non-SGS samples decreased, making their  $L^*$  values comparable. All samples had decreased lightness at day 21 and no more significant changes observed for longer chilled storage.

Additional processing with high pressure (HPP and SGS-HPP) and microwave (MVH and SGS-MVH) did not significantly change the lightness, redness and yellowness compared to non-additional processing (VAC and SGS-VAC). Lightness and yellowness of HPP chicken were more changed during storage than redness, i.e.  $L^*$  value significantly decreased and  $b^*$  value significantly increased at day 119 compared to day 0. The minimal/no effect of HPP on redness of cooked chicken during storage in the present study was in line with several earlier reported findings on meat products (Hygreeva et al., 2017; Mor-Mur and Yuste, 2003). It has been reported that the HPP effect on color was more pronounced in fresh meat than in cooked meat since HPP causes myoglobin (the major pigment in chicken meat) denaturation, oxidation of ferrous myoglobin to ferric metmyoglobin, and pressure-induced changes in other proteins (Mor-Mur and Yuste, 2003; Simonin et al., 2012). The present findings might support the potential of HPP and MVH in long-term preservation of precooked chicken without compromising the color quality.

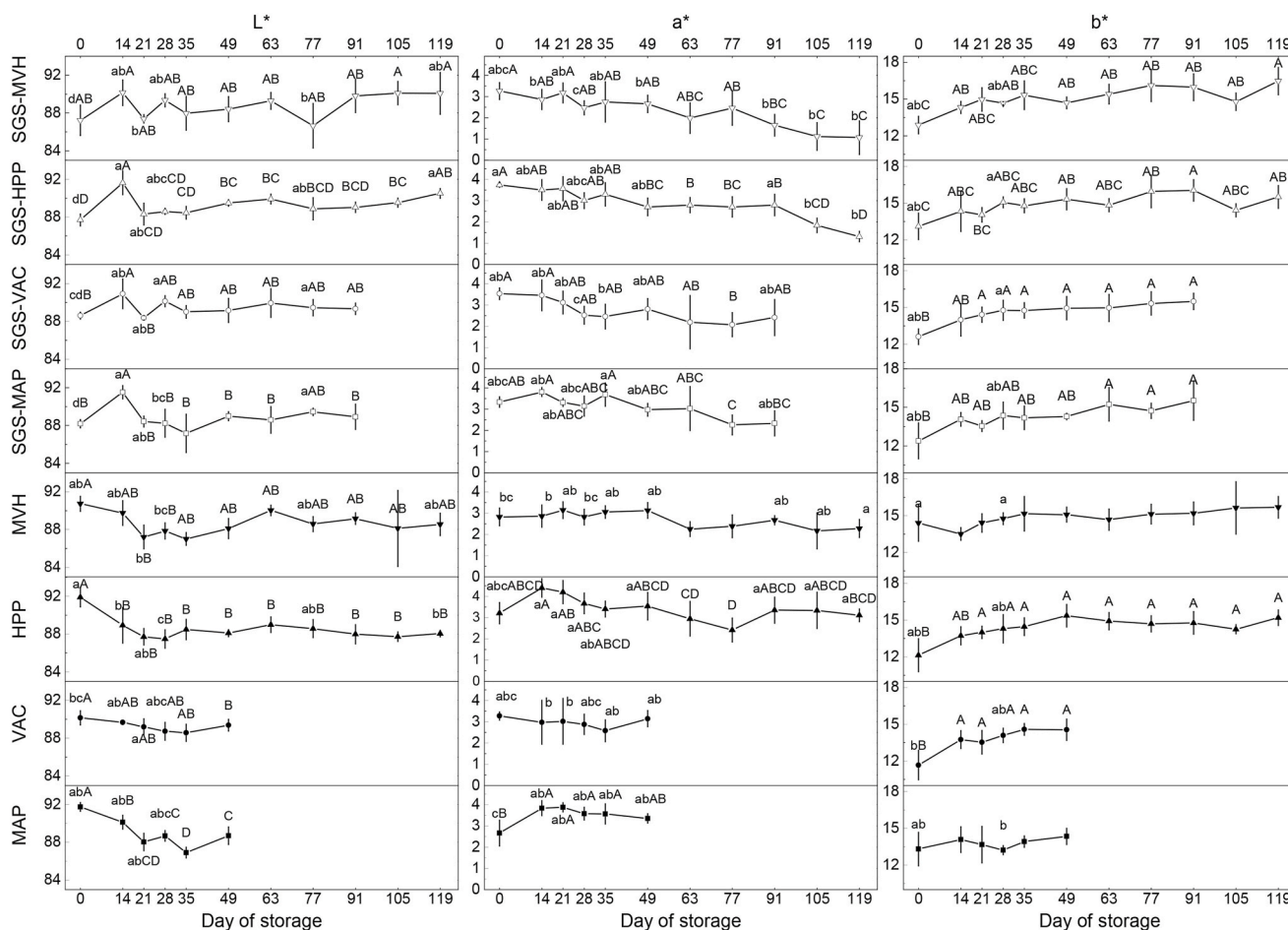


Fig. 3. The effect of storage duration and varying treatments on the color of precooked chicken. Each point is a mean of 5 replicates. Small letters (a, b, c) indicate significant difference ( $p < 0.05$ ) between treatments at the same time point, and capital letters (A, B, C) indicate significant difference ( $p < 0.05$ ) between days of storage in a treatment. Error bars indicate standard deviations.

### 3.3. Drip loss

The effect of treatments and storage on drip loss of precooked chicken slices is presented in Fig. 4. Most treatments did not significantly change the drip loss (~3%) during storage, except for MVH and SGS-MAP which slightly increased over storage time. The low amount and stability of drip loss during refrigerated storage may indicate that either the studied treatments were mild or the cooking before the treatments already caused most of the drip loss thus low drip loss was observed at further processing. Kong et al. (2008) reported that during heating at 121 °C, most drip loss occurred during the first 20 min (26.2%), and longer cooking did not significantly change the drip loss. In the present study, the chicken breast was cooked for total 26 min at 125–130 °C (oven) and the average drip loss of chicken fillets was 22.8 ± 3.1%. Drip loss in cooking is a result of mainly water loss from denaturation of myofibrillar and sarcoplasmic proteins, reducing water holding capacity (Fernandez et al., 2007; Iwasaki et al., 2006). After cooking, the denatured sarcoplasmic, myofibrillar proteins and melted collagen might form an aggregate gel that reduced drip loss in further processing (Kong et al., 2008). Also, there was no statistically significant difference between the eight treatments at day 0, but treatments were more differentiated during storage, showing that more changes of muscle structure

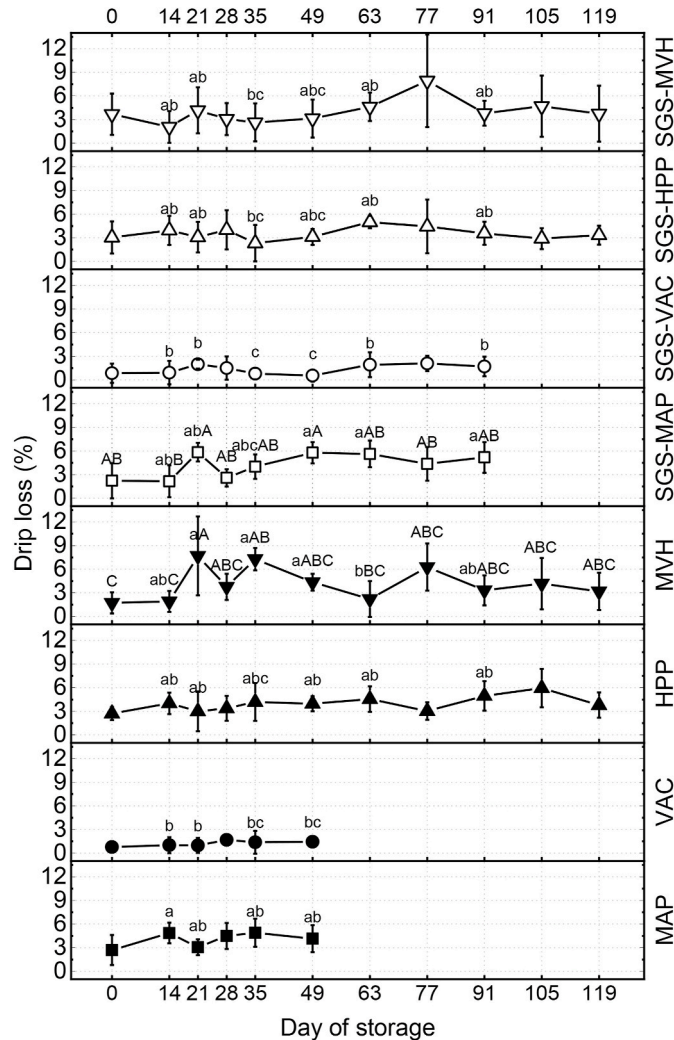


Fig. 4. The effect of storage duration and varying treatments on the drip loss of precooked chicken. Each point is a mean of 5 replicates. Small letters (a, b, c) indicate significant difference ( $p < 0.05$ ) between treatments at the same time point, and capital letters (A, B, C) indicate significant difference ( $p < 0.05$ ) between days of storage in a treatment. Error bars indicate standard deviations.

occurred in some treatments (e.g. MVH, MAP, SGS-MAP) than others. In comparison between two packaging techniques, MAP resulted in higher drip loss than VAC, being significant from day 14 in non-SGS samples, and day 49 in SGS samples. This was similar to MAP-packed beef that had greater drip loss than VAC-packed beef during refrigerated storage (Zakrys-Waliwander et al., 2012). Okeeffe and Hood (1981) reported that CO<sub>2</sub> had negative effect on drip loss since it caused pH reduction, consequently resulting in low water holding capacity.

### 3.4. Microbiology

Prior to studying the effect of processing and packaging on microbial growth of precooked chicken, the total viable count (TVC), lactic acid bacteria (LAB), *E. coli*, *Listeria* and *Campylobacter* of raw chicken were quantified. The raw chicken was found to be contaminated with 4.0 ± 0.3 log CFU/g of TVC, 3.1 ± 0.5 log CFU/g of LAB, 1.8 ± 0.5 log CFU/g of *E. coli*, 1.3 ± 0.3 log CFU/g of *Listeria* and no detection of *Campylobacter*. After treatments (day 0) and during storage, pathogens *E. coli*, *Campylobacter* and *Listeria* were below detection limit (<1 log CFU/g for *E. coli*, < 1.3 log CFU/g for *Campylobacter* and *Listeria*) in all samples. Therefore, only TVC and LAB of precooked chicken slices treated with different processing and packaging methods during chilled storage are presented in Table 1. The population of TVC and LAB was almost similar, indicating that most of TVC present in precooked chicken slices were lactic acid bacteria. Both treatments and storage had significant effect on TVC and LAB population. Samples with additional processing like HPP, MVH, SGS-HPP and SGS-MVH were effective in microbial shelf life extension to certain extent. HPP and SGS-HPP were the most efficient treatments to extend the shelf life of chicken slices. HPP impressively remained TVC and LAB close to the detection limit (1.3 log CFU/g) until day 119, and SGS-HPP remained until day 105 before some growth shown on day 119. Although MVH and SGS-MVH showed some marginal inactivation of TVC and LAB compared to their corresponding controls VAC and SGS-VAC, uneven heating from microwave caused large variation between replicates at some sampling points. With respect to packaging, MAP-packed chicken showed relatively higher bacterial load than VAC-packed chicken, and the use of SGS prior to packaging improved the TVC and LAB inhibition. However, bacterial load varied substantially in SGS treated samples, except for SGS-HPP, suggesting that CO<sub>2</sub> might be absorbed unequally throughout the chicken fillet, consequently cuts from the fillets might contain different amount of CO<sub>2</sub> and bacteria.

The effect of HPP on microbial inactivation and on stability of low bacterial level during storage has consistently been reported in chicken products (Andreou et al., 2018; Kruk et al., 2011; Luckose et al., 2015; Rodriguez-Calleja et al., 2012). The underlying mechanism could be related to the pressure-induced cell membrane damage resulting in the leakage of ATP from the cell which finally lead to cell death (Smelt et al., 1994). Also, the SGS results from the present study were in line with previous studies on chicken (Al-Nehlawi et al., 2013; Rotabakk et al., 2006). Formation of carbonic acid and/or detrimental effects of CO<sub>2</sub> on enzymatic and biochemical pathways might be responsible for retarding bacteria during SGS (Daniels et al., 1985).

### 3.5. pH

The effect of treatments and storage on pH of precooked chicken slices is presented in Fig. 5. There were only minor changes in pH ranging from 6.01 to 6.34 for all treatments during storage. Almost no difference in pH between the control and novel processed samples (VAC vs HPP and MVH, SGS-VAC vs SGS-HPP and SGS-MVH) at most of sampling time were observed. This reveals that novel processing did not affect pH of precooked chicken during chilled storage. This result was in agreement with previous studies, that HPP and MVH did not affect the pH of chicken (Siddig et al., 2019; Taskiran et al., 2020). pH of SGS chicken was similar to pH of non-SGS chicken once the chicken was

**Table 1**

The effect of storage duration and varying treatments on the microorganisms of precooked chicken (log CFU/g).

Day of storage	MAP	VAC	HPP	MVH	SGS-MAP	SGS-VAC	SGS-HPP	SGS-MVH
<b>TVC</b>								
0	1.3 ± 0.0 <sup>abC</sup>	1.3 ± 0.3 <sup>abB</sup>	1.2 ± 0.2 <sup>ab</sup>	1.3 ± 0.3 <sup>ab</sup>	2.0 ± 0.6 <sup>aAB</sup>	1.1 ± 0.2 <sup>b</sup>	1.0 ± 0.0 <sup>BB</sup>	1.0 ± 0.0 <sup>b</sup>
14	5.5 ± 0.6 <sup>abB</sup>	6.4 ± 0.7 <sup>aA</sup>	1.1 ± 0.2 <sup>b</sup>	1.0 ± 0.0 <sup>b</sup>	2.7 ± 1.6 <sup>baB</sup>	1.0 ± 0.0 <sup>b</sup>	1.0 ± 0.0 <sup>BB</sup>	1.6 ± 1.0 <sup>b</sup>
21	6.2 ± 0.2 <sup>aAB</sup>	5.8 ± 1.5 <sup>aA</sup>	1.1 ± 0.2 <sup>b</sup>	3.7 ± 2.2 <sup>ab</sup>	5.8 ± 0.7 <sup>aAB</sup>	2.8 ± 2.1 <sup>ab</sup>	1.1 ± 0.2 <sup>BB</sup>	2.7 ± 2.4 <sup>ab</sup>
28	6.7 ± 0.1 <sup>abA</sup>	7.6 ± 0.4 <sup>aA</sup>	1.2 ± 0.3 <sup>c</sup>	7.1 ± 0.8 <sup>a</sup>	2.5 ± 0.3 <sup>bcAB</sup>	4.7 ± 3.2 <sup>abc</sup>	1.0 ± 0.0 <sup>CB</sup>	2.6 ± 2.9 <sup>bc</sup>
35	6.9 ± 0.5 <sup>abA</sup>	7.6 ± 0.4 <sup>aA</sup>	1.0 ± 0.0 <sup>c</sup>	4.0 ± 2.6 <sup>bc</sup>	1.0 ± 0.0 <sup>CB</sup>	4.8 ± 2.3 <sup>ab</sup>	1.0 ± 0.0 <sup>CB</sup>	1.1 ± 0.2 <sup>c</sup>
49			1.9 ± 1.6	7.0 ± 1.5	5.0 ± 3.5 <sup>AB</sup>	2.6 ± 1.7	1.3 ± 0.5 <sup>AB</sup>	2.3 ± 2.3
63			1.0 ± 0.0 <sup>b</sup>	6.7 ± 2.0 <sup>a</sup>	3.9 ± 2.9 <sup>abAB</sup>	1.1 ± 0.2 <sup>a</sup>	1.0 ± 0.0 <sup>AB</sup>	1.8 ± 1.3 <sup>a</sup>
77			1.0 ± 0.1	3.4 ± 3.1	5.2 ± 3.7 <sup>A</sup>	3.5 ± 4.4	1.0 ± 0.0 <sup>B</sup>	3.3 ± 3.9
91			1.3 ± 0.5 <sup>ab</sup>	3.2 ± 3.8 <sup>ab</sup>	7.3 ± 1.3 <sup>aA</sup>	3.1 ± 3.6 <sup>ab</sup>	1.0 ± 0.0 <sup>BB</sup>	1.3 ± 0.0 <sup>ab</sup>
105			1.0 ± 0.0	2.5 ± 2.6			1.0 ± 0.0 <sup>B</sup>	4.3 ± 3.3
119			1.0 ± 0.0	1.0 ± 0.0			4.0 ± 3.1 <sup>A</sup>	1.0 ± 0.0
<b>LAB</b>								
0	1.0 ± 0.0 <sup>C</sup>	1.0 ± 0.0 <sup>B</sup>	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0 <sup>B</sup>	1.0 ± 0.0
14	5.5 ± 0.6 <sup>abB</sup>	6.3 ± 0.6 <sup>aA</sup>	1.0 ± 0.0 <sup>b</sup>	1.0 ± 0.0 <sup>b</sup>	2.8 ± 1.6 <sup>b</sup>	1.0 ± 0.0 <sup>b</sup>	1.0 ± 0.0 <sup>BB</sup>	1.6 ± 1.0 <sup>b</sup>
21	6.2 ± 0.2 <sup>aAB</sup>	5.7 ± 1.5 <sup>aA</sup>	1.0 ± 0.0 <sup>b</sup>	2.6 ± 2.7 <sup>ab</sup>	5.8 ± 0.8 <sup>a</sup>	2.4 ± 2.4 <sup>ab</sup>	1.0 ± 0.0 <sup>BB</sup>	2.5 ± 2.5 <sup>ab</sup>
28	6.7 ± 0.1 <sup>abA</sup>	7.6 ± 0.4 <sup>aA</sup>	1.0 ± 0.0 <sup>c</sup>	4.8 ± 3.4 <sup>abc</sup>	1.8 ± 1.3 <sup>bc</sup>	4.7 ± 3.2 <sup>abc</sup>	1.0 ± 0.0 <sup>CB</sup>	2.6 ± 2.9 <sup>abc</sup>
35	6.9 ± 0.6 <sup>aA</sup>	7.7 ± 0.3 <sup>aA</sup>	1.0 ± 0.0 <sup>b</sup>	2.6 ± 2.7 <sup>b</sup>	1.0 ± 0.0 <sup>b</sup>	1.0 ± 0.0 <sup>b</sup>	1.0 ± 0.0 <sup>BB</sup>	1.0 ± 0.0 <sup>b</sup>
49			1.9 ± 1.6	1.0 ± 0.0	5.1 ± 3.6	1.1 ± 0.2	1.0 ± 0.0 <sup>B</sup>	1.0 ± 0.0
63			1.0 ± 0.0	2.4 ± 2.5	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0 <sup>B</sup>	1.7 ± 1.2
77			1.0 ± 0.1	3.0 ± 3.4	5.1 ± 3.6	1.0 ± 0.0	1.0 ± 0.0 <sup>B</sup>	3.2 ± 3.8
91			1.0 ± 0.0	1.0 ± 0.0	2.9 ± 3.2	2.8 ± 3.1	1.0 ± 0.0 <sup>B</sup>	1.0 ± 0.0
105			1.0 ± 0.0	1.9 ± 1.6			1.0 ± 0.0 <sup>B</sup>	3.2 ± 3.7
119			1.0 ± 0.0	1.0 ± 0.0			4.0 ± 3.1 <sup>A</sup>	1.0 ± 0.0

Each value in the table is the mean ± standard deviation of 3 replicates. Small letters (a, b, c) indicate significant difference ( $p < 0.05$ ) between treatments at the same time point (same row), and capital letters (A, B, C) indicate significant difference ( $p < 0.05$ ) between days of storage in a treatment (same column).

subsequently vacuum packed but significantly lowered once the chicken was subsequently MAP packed. This indicates that carbonic acid formation from SGS was insufficient to significantly change pH of precooked chicken but was sufficient when combined with subsequent MAP packaging. VAC- and MAP-based samples tended to increase pH in the first 21 and 14 d respectively before declining gradually over remaining course of storage. The pH increase during the first 14–21 d could be related to the accumulation of ammonia and products of amino acid decomposition during storage (Masniyom et al., 2002). Most of ammonia formed originates from enzymatic deamination of amino acids, from oxidation of amines and from decomposition of nucleic bases (Huang et al., 1993). The slight pH decrease after 21 d in VAC, SGS-VAC, and SGS-MAP could be attributed to increased population of lactic acid bacteria that produced lactic acid and lowered the pH of chicken (Table 1).

### 3.6. Lipid oxidation

Lipid oxidation is one of the causes of quality deterioration in meat and meat products (Dominguez et al., 2019). The effect of different processing and packaging treatments and storage on lipid oxidation of chicken slices is shown in Fig. 6A. Both treatment and storage time significantly affected the lipid oxidation of precooked chicken slices. For all treatments, lipid oxidation substantially increased over the entire storage time, except for a decline in VAC and SGS-VAC samples after 49 d of storage. HPP and MVH treated samples oxidized least compared to their control (VAC) and other samples at all sampling time. Particularly, immediately after processing (day 0), both HPP and MVH significantly ( $p < 0.05$ ) lowered the malondialdehyde (MDA) amount by almost half compared to their common control (VAC), and this lowering pattern remained throughout the storage. This indicates that HPP and MVH partly prevented the lipid oxidation. The result of HPP treated samples was in accordance with several previous findings (Hygreeva et al., 2017; Rakotondramavo et al., 2019) which explained that HPP may produce compounds capable of reacting with MDA, consequently lower available MDA content. It appeared that HPP affected lipid oxidation of precooked meat in a way differently from its effect on lipid oxidation of non-precooked meat. Particularly, HPP decreased or unchanged lipid

oxidation in precooked meat as seen in Fig. 6 and in previous studies (Hygreeva et al., 2017; Rakotondramavo et al., 2019) but increased in non-precooked meat and the increase is more marked with higher pressure level (Ma et al., 2007; Orlien et al., 2000). This phenomenon suggests that thermal treatment might trigger more oxidative reaction than might high pressure do. Indeed, the MDA content and its increase rate during refrigerated storage induced by conventional heating (80 °C, 10 min) was higher than by high pressure (300–800 MPa, 5–10 min) as reported by Orlien et al. (2000). In the present study, the material subjected to HPP was precooked (90 °C core temperature) chicken, it was therefore not surprising that HPP reduced rather than stimulated the oxidative product (MDA).

Also seen from Fig. 6, SGS pretreatment instantly suppressed MDA, but promoted a greater development rate of MDA during refrigerated storage. Particularly, MDA content of SGS-VAC (0.13 mg/kg), SGS-MAP (0.17 mg/kg), and SGS-HPP (0.28 mg/kg) at day 0 were significantly lower than the corresponding MDA content of VAC (0.47 mg/kg), MAP (0.62 mg/kg) and HPP (0.29 mg/kg). Throughout storage, MDA of these SGS treated chicken developed at a faster rate than corresponding non-SGS chicken, e.g. 2.94–3.5 vs 2.68–2.81 at day 28; 2.96–3.52 vs 2.8–2.96 at day 49 etc. (Fig. 6B). Probably like HPP, CO<sub>2</sub> gas stabilization generated compounds that temporarily reacted with pre-cooking-induced oxidative products, making MDA value low at day 0. The oxidative products were nevertheless released gradually, combined with newly generated MDA showing higher values at day 28–105. The oxidation suppression of SGS may be also caused by absolute absence of oxygen immediately after cooking, stopping the secondary oxidation stage in which primary oxidative products (e.g. hydroperoxides) naturally react with oxygen to form secondary oxidative products (e.g. MDA).

Both VAC and MAP in the present were oxygen absent packaging methods, but lipid oxidation of VAC-packed chicken at day 0 was lower than that of MAP-packed chicken (0.47 vs 0.62 mg MDA/kg). This shows that vacuum withdrew not only air inside the package but also oxygen inside VAC-packed chicken slice, consequently inhibiting some ongoing oxidation process.



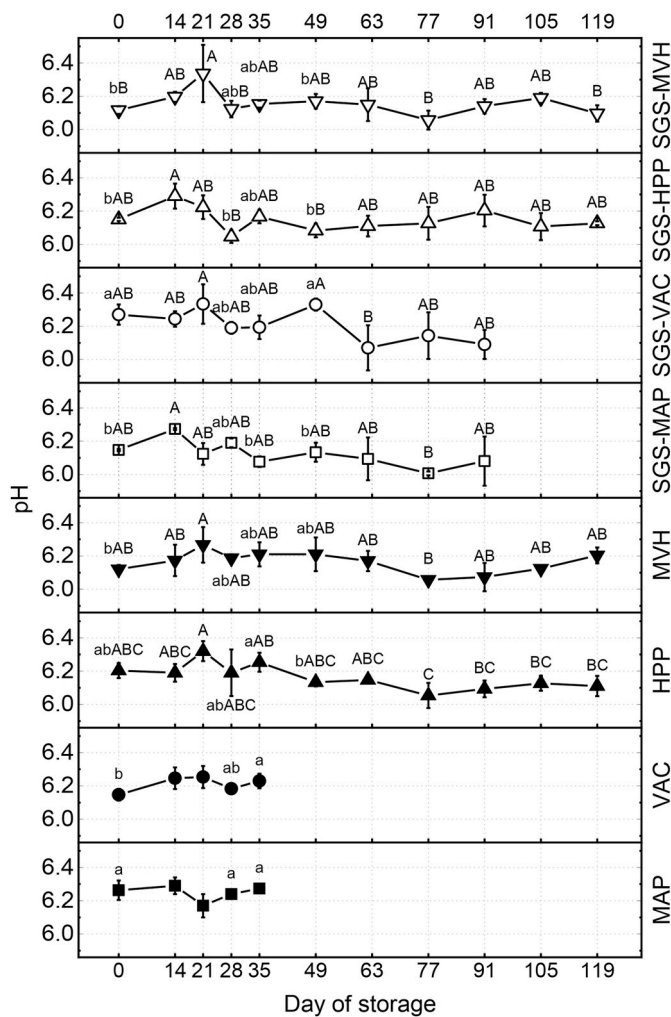


Fig. 5. The effect of storage duration and varying treatments on the pH of precooked chicken. Small letters (a, b, c) indicate significant difference ( $p < 0.05$ ) between treatments at the same time point, and capital letters (A, B, C) indicate significant difference ( $p < 0.05$ ) between days of storage in a treatment. Error bars indicate standard deviations.

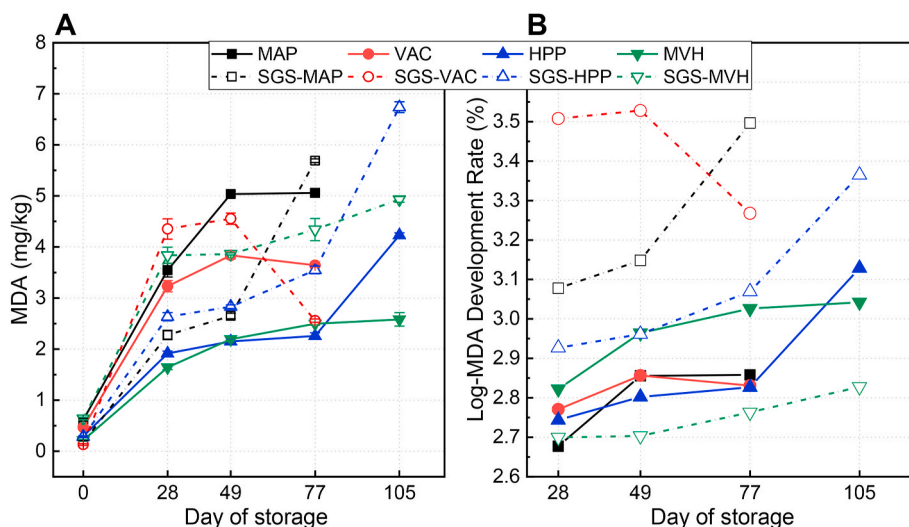


Fig. 6. The effect of storage duration and varying treatments on the lipid oxidation of precooked chicken (A) and the development rate expressed as log ((MDA day n - MDA day 0)\*100/MDA day 0) during refrigerated storage (B). Error bars indicate standard deviations.

### 3.7. Correlation between parameters

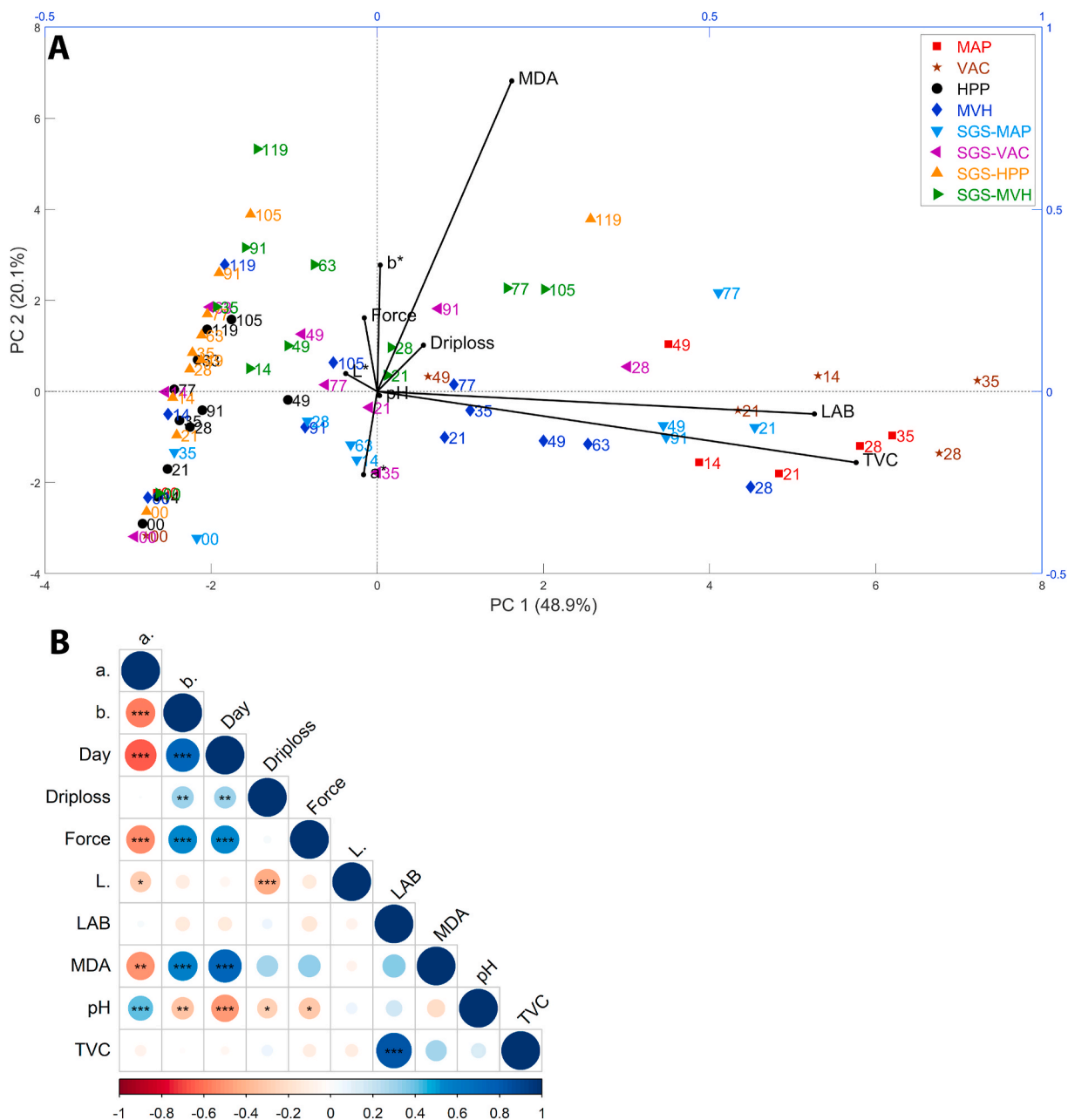
The PPCA was used as an exploratory tool to visualize grouping trends of samples and variables. The data matrix comprised 74 samples and 8 independent variables (TCA, LAB, drip loss, pH, MDA,  $L^*$ ,  $a^*$ , and  $b^*$ ). The biplot of the first two principal components of the samples and variables explained 69% of the variance (Fig. 7A). The most prominent quality attributes were TVC count, LAB count and MDA content on the first PC. MAP and VAC from day 14, SGS-MAP from day 21 and SGS-VAC from day 28 were associated with highest TVC and LAB growth. MVH from day 21 and SGS-MVH from day 78 had relatively medium TVC and LAB growth. All samples at day 0, all HPP from day 0–119, all SGS-HPP from day 0 to day 105 were separated from TVC and LAB, meaning that they had very low level of those bacteria. However, they were associated with  $L^*$ ,  $a^*$  and force, showing that they were lighter, more red, and harder than other samples.

The Pearson's correlation coefficients with statistical significance between storage time and quality attributes is shown in Fig. 7B. As seen, storage time (day) was significantly positively correlated with  $b^*$ , drip loss, force, and MDA content, while it was negatively correlated with pH and  $a^*$  value. As TVC and LAB were highly dependent on individual treatments, the overall correlation between these quality parameters were not clearly established with storage day and other quality parameters. pH showed a negative correlation with force, drip loss, and  $b^*$  value, and a positive correlation with  $a^*$  value. Extent of lipid oxidation expressed as MDA level was negatively correlated with  $a^*$  and pH, but positively correlated with  $b^*$ , drip loss, force, and TVC. The negative interrelationship between lipid oxidation and redness was also seen in raw chilled broiler breast meat (Viana et al., 2017), goat loin meat (Kannan et al., 2001), beef, pork, and turkey meat (Akamittath et al., 1990). These results suggested that pigment oxidation can catalyze lipid oxidation, and vice versa.

### 4. Conclusion

Shelf life of precooked chicken breast slices subjected to modified atmosphere packaging (40% CO<sub>2</sub>, 60% N<sub>2</sub>), vacuum packaging (93%), high pressure (600 MPa, 2 min), microwave (1 kW, 3 × 15 s), and soluble gas stabilization (100% CO<sub>2</sub>, 18 h, 1 °C) was evaluated. HPP with or without combination with SGS was the most effective method extending the shelf life of precooked chicken to more than 3 months as compared to 28 d of vacuum-packed chicken while maintaining the physico-chemical quality characteristics. Short-time MVH appeared to be





**Fig. 7.** Probabilistic principal components analysis (PPCA) biplot of total 74 sliced-chicken samples (8 treatments with various days of storage) and 8 quality variables (A), and correlation of variables (B). Treatments are symbolized and colored uniquely. Samples at particular sampling day are separated in the scores plot (filled symbols). The loadings (black projected lines) shows the quality variables responsible for the separation.

effective but occasional uneven heating influenced the consistency of microbial load between sample replicates. SGS was a promising pre-treatment prior to packaging in reducing the bacterial population but tended to temporarily turn the chicken darker immediately after pre-treatment before returning to normal color in two weeks of storage. We suggest that sensory assessment should be conducted in future studies to evaluate the overall quality of well extended shelf life chicken. The findings from the present study can benefit ready-to-eat chicken processors in different ways: providing alternative shelf life extending methods, comparisons between them for shelf life and other quality balance, finding out the relationship between quality parameters from which one can correlate a desired parameter based on others.

**CRedit authorship contribution statement**

**Tem Thi Dang:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Tone Mari Rode:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision. **Dagbjørn Skjpnæs:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision.

**Declaration of competing interest**

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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jfoodeng.2020.110352>.

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