

RESEARCH ARTICLE

# Combined effect of carvacrol and high hydrostatic pressure on quality attributes of chicken meat during refrigerated conditions

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## ABSTRACT

The application of hurdle approach using high hydrostatic processing (HHP) with bioactive compounds (BACs) to preserve meat quality is promising due to safety, improved well-being, and easier acceptance by customers compared to the use of synthetic preservatives. This study was designed to investigate the influence of natural phenolic BAC carvacrol (CARV) at 500 and 1000 ppm combined with HHP (300 and 600 MPa) on the quality attributes of chicken meat after being vacuum-sealed and kept at  $4 \pm 0.5$  °C for 28 days. The HHP showed a significantly higher pH rate ( $P < 0.05$ ), increased  $L^*$  and  $b^*$  value, decreased  $a^*$  values, and reduction in water holding capacity (WHC) compared to unpressurized control meat with/without CARV. Reduction in thiobarbituric acid reactive substances (TBARS) indexes was perceived in meat supplemented with CARV, and low level of HHP (300 HHP) whereas significantly increased lipid oxidation was witnessed with HHP 600 MPa. At day 28, an increase in aerobic mesophilic counts (AMCs) was observed in all meat samples. However, reductions of about 0.6, 0.8, and 1.1 log in AMCs were seen in meat treated with 600HHP-No CARV, HHP300-CARV 500 ppm, and 600HHP-CARV 1000 ppm, respectively. Furthermore, despite the overlapping aroma pattern between meat containing CARV and control groups, the electronic nose was able to discriminate control from samples subjected to HHP. The current results demonstrate that the addition of CARV improved the conservation effects of HHP with less oxidative deterioration of fresh chicken meat during chilling storage.

**Keywords:** Bioactive compounds; High hydrostatic processing; Chicken meat; Lipid oxidation; Microbiological properties

## INTRODUCTION

The extension of shelf-life of meat and meat products is majorly dependent on the consecutive control of conditions including microbial decomposition, processes of oxidation, and organoleptic changes under room and chilled storage circumstances. Chicken meat perishable in nature provides an almost perfect medium for microbial contamination of both spoilage and pathogenic microorganisms that lead to food safety and public health issues (Karabagias et al., 2011). Oxidative rancidity is another key cause of quality deterioration of fresh poultry meat during refrigerated and frozen storage attributed to its high content of polyunsaturated fatty acids (Barroeta 2007). In addition, sensorial changes are also attributed to the potential causes

of quality deterioration in meat. The proteins/lipids degradation can lead to unpleasant quality properties (such as discolouration, less water holding capacity, texture with soft and exudative characteristics, and off flavour/odour) and eventually influence product acceptance by consumers (Lucera et al., 2012). Therefore, there is a growing demand of consumers for products with high sensorial quality, and the application of the hurdle concept is in the potential to produce food products in a safe and natural manner to meet the demand of consumers.

High hydrostatic processing (HHP) is an emerging non-thermal method used in food preservation and food processing. This technique is being applied to extend the shelf-life and preserve the quality of meat and meat

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products that have been processed, followed by a minimal effect on the sensorial and nutritional quality of attributes (Marcos et al., 2013). In HHP technique, the food is subjected to pressure that is intense with loads of about 1000 MPa, consuming a fluid with non-compressible pressure-transmitting features. In order to prevent the mechanisms of the degradation process in food to occur, pressure loads applied are relatively moderate to intense which results in inactivating the deteriorative enzymes and eliminating the pathogenic and spoilage microorganisms (Rendueles et al., 2011). Moreover, it has been claimed that the main site of action for HHP in bacteria is the cytoplasmic membrane, but the precise cellular mechanisms that cause damage have not been elucidated and might be complex. Additionally, recovery of function can occur in microorganisms that showed several sub-lethally injuries, especially during a favourable storage conditions (Ait-Ouazzou et al., 2013). Furthermore, HHP can have an effect on various meat characteristics including structure, morphology, and physicochemical, and may cause fresh red meat to partially discolour (Kim et al., 2007). Consequently, the application of HHP combined with an additional hurdle could lead to a synergistic or additive effect to enhance the preservation impact of pressurized food. Essential oil has been widely used in meat preservation particularly to control spoilage and lipid oxidation (Erkan et al., 2010; Makri, 2013). Moreover, using natural bioactive compounds (BACs) as bio-preservatives to improve the meat quality is promising since they are considered as GRAS (Generally Recognized as Safe) and due to easier acceptance by customers compared to synthetic preservatives (Amiri et al., 2021).

CARV (5-isopropyl-2-methylphenol), derived flavoured phenolic BAC-a exists as a main component in oregano, thyme, sage, and savoury. The antimicrobial and antioxidant activity has been shown by this BAC (Ultee, 2002; Hussein et al., 2019). It has been studied that CARV showed antimicrobial effects against several food spoilage and pathogenic microorganisms including *Pseudomonas* spp, *Staphylococcus aureus*, *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Campylobacter jejuni*. In addition, CARV showed antioxidant activity, resulting from the donation of hydrogen atoms/electrons attributed to the aromatic ring particularly their hydroxyl groups eventually helping to stabilize free radicals (Hernández-Ochoa et al., 2014; Kim et al., 2013; Zengin and Baysal, 2014). It was witnessed that the information that exists is limited, especially on the quality characteristics of chicken meat treated with a combination of natural food additives and HHP. Therefore, this research aimed to investigate a simultaneous combined effect of natural phenolic BACs (carvacrol) at 500 and 1000 ppm and HHP (300 and 600 MPa), particularly on the microbiological (aerobic mesophilic counts-AMCs),

lipid oxidation (TBARS), physicochemical attributes and sensory (Electronic nose) aspects in fresh, minced chicken meat that has been vacuum packaged.

## MATERIALS AND METHODS

### Preparation of meat samples

The chicken breast meat used in this study received fresh 24-hour post-mortem from a local abattoir and was brought to the research laboratory in chilling conditions. The skin of the meat was removed, and the meat was minced using a meat grinder (BOSCH - Slovenia) equipped with 3 grinding plates then homogenized and the meat was divided into various groups. The treated meat samples were mixed with CARV (500 and 1000 ppm) per weight of meat (dissolved in 5% sunflower oil); whereas no CARV was added to the group of control. The BAC (CARV) (98%) was obtained from (SIGMA-Germany.) The meat samples were sealed (Multivac-Germany) in polyethylene bags and vacuum packaged before being subjected to HHP.

### High hydrostatic pressure treatment

The polyethylene bag that contains meat samples was then vacuum packed and placed in a vessel that pressurized for 5 minutes with 300 and 600 MPa in a RESATO-FPU 100-2000 HHP-unit (Resato International B.V., Netherlands). On days 1, 14, and 28, the chicken meat samples were collected for measurements while being kept at  $4 \pm 0.5$  °C.

### Physicochemical properties

The measurement of meat pH was carried out using Testo pH meter (Testo 206; Titisee-Neustadt, Germany) by immersing a pH electrode in triplicate into the minced meat samples.

The colour values of chicken meat:  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) were measured due scoring system known as CIELAB (CIE, 1986) by applying colourimeter Chroma Meter CR-400 (Konica-Minolta Sensing Inc., Japan).

The water holding capacity (WHC) was analysed in triplicates by applying the filter paper press technique applied by Grau & Hamm (1953).

### Measurement of TBARS

The TBARS values in meat were measured as an indicator of lipid oxidation by using the technique applied by Tarladgis et al. (1960). The values of TBARS were presented as milligrams of malondialdehyde (MDA equivalent) per kilogram of meat samples (De Oliveira et al., 2015).

### Microbiological analysis

The aerobic mesophilic counts (AMCs) were measured as indicators of the microbiological status of chicken

meat using the procedure described by Jridi et al. (2015). The AMC's results were presented as log CFU/g (log: logarithms) (CFU: colony forming unit) per g of meat.

**Electronic nose determination**

The analyses of the electronic nose were carried out by the technique applied by Dalmadi et al. (2007) using the NST-3320 apparatus (Applied Sensor-Technologies, Sweden). The change in sensor signals after the sampling time ends, among the baseline and the value signals were used as a sensor response for multivariate statistical analysis, and to distinguish between various meat groups canonical discrimination analysis (CDA) was used.

**STATISTICAL ANALYSIS**

The result was presented as mean and standard deviation. The software SPSS (Version 23.0, SPSS Inc.) was applied to analyse the experimental data using analysis of variance (ANOVA) and General Linear Model (GLM), and Tukey test was applied to describe the degree of significance ( $P < 0.05$ ).

**RESULTS AND DISCUSSION**

**Physicochemical properties**

In meat samples treated with 300 and 600 MPa of HHP, the pH values showed a significantly higher rate compared to unpressurized meat control with/without CARV ( $P < 0.05$ ). The different concentrations of CARV did not show a significant difference in samples treated with/without HHP compared to the control (Table 1). On day 28 of storing and compared to day one of storing a significant reduction of pH was noticed in all samples except the sample treated with 600 MPa HHP that did not contain CARV. De Oliveira et al. (2015) used CARV and HHP in sliced vacuum-packed turkey breast ham with low-sodium, similar to the current findings they observed a reduction in the rate of pH values which slightly differ between the various treatments assessed for 30 days stored in refrigerated conditions. It is known that a rise in the pH levels can be produced in pressurized meat and meat products, which may be the result of denaturation caused by a reduction in acidic groups attributable to conformational variations in the proteins.

During the storage period, significant differences with intensifying trends in lightness ( $L^*$ ) were noticed in chicken meat treated with 600 MPa HHP compared to 300 MPa HHP and compared to unpressurized meat in control with/without CARV (Table 1). The higher concentration of CARV (1000-ppm) showed significantly higher  $L^*$  compared to untreated meat. Similarly, an increase

**Table 1: Effect of various concentrations of CARV and HHP levels (300 and 600 MPa) on physicochemical characteristics of fresh chicken meat stored at 4°C**

Parameters	Storage (d)	Control			CARV+300HHP			CARV+600HHP		
		No-CARV	CARV-500ppm	CARV-1000ppm	No-CARV	CARV-500ppm	CARV-1000ppm	No-CARV	CARV-500ppm	CARV-1000ppm
pH	1	5.96±0.01 <sup>aAZ</sup>	5.94±0.01 <sup>aAZ</sup>	5.94±0.02 <sup>aAY</sup>	6.02±0.01 <sup>BAZ</sup>	6.02±0.00 <sup>BAZ</sup>	6.02±0.02 <sup>BAZ</sup>	6.12±0.01 <sup>CAZ</sup>	6.11±0.01 <sup>CAZ</sup>	6.11±0.01 <sup>CAZ</sup>
	14	5.77±0.01 <sup>aAX</sup>	5.77±0.01 <sup>aAX</sup>	5.76±0.01 <sup>aAX</sup>	5.81±0.01 <sup>BAZ</sup>	5.81±0.02 <sup>BAZ</sup>	5.84±0.01 <sup>BAZ</sup>	6.07±0.02 <sup>CAZ</sup>	6.07±0.01 <sup>CAZ</sup>	6.09±0.01 <sup>CAZ</sup>
	28	5.86±0.01 <sup>aAY</sup>	5.84±0.01 <sup>aAY</sup>	5.87±0.05 <sup>aAY</sup>	5.78±0.01 <sup>aAX</sup>	5.82±0.01 <sup>aAX</sup>	5.81±0.01 <sup>aAX</sup>	6.08±0.02 <sup>CAZ</sup>	6.02±0.05 <sup>CAZ</sup>	5.99±0.07 <sup>aAX</sup>
$L^*$	1	49.62±0.30 <sup>aAX</sup>	49.48±0.23 <sup>aAX</sup>	49.63±0.42 <sup>aAX</sup>	67.92±0.58 <sup>BAZ</sup>	68.76±0.8 <sup>BAZ</sup>	69.14±0.57 <sup>BAZ</sup>	78.32±0.61 <sup>CAZ</sup>	78.08±0.63 <sup>CAZ</sup>	79.05±0.43 <sup>CAZ</sup>
	14	50.98±0.36 <sup>aBY</sup>	49.87±0.58 <sup>aAX</sup>	50.70±0.31 <sup>aBY</sup>	68.85±0.91 <sup>BAZ</sup>	68.10±0.38 <sup>BAZ</sup>	68.36±1.05 <sup>BAZ</sup>	78.15±0.53 <sup>CAZ</sup>	76.31±1.56 <sup>CAZ</sup>	76.99±0.50 <sup>CAZ</sup>
	28	52.27±0.70 <sup>aAZ</sup>	52.36±0.77 <sup>aAY</sup>	54.00±0.49 <sup>aBZ</sup>	69.86±0.51 <sup>BAZ</sup>	69.68±0.50 <sup>BAZ</sup>	70.73±0.6 <sup>BAZ</sup>	77.07±0.14 <sup>CAZ</sup>	77.94±0.39 <sup>CAZ</sup>	78.43±0.19 <sup>CAZ</sup>
$a^*$	1	4.44±0.38 <sup>aBAX</sup>	4.43±0.37 <sup>aBAX</sup>	4.35±0.21 <sup>aAX</sup>	5.29±0.78 <sup>BAZ</sup>	4.63±0.37 <sup>BAZ</sup>	4.36±0.46 <sup>BAZ</sup>	3.62±0.22 <sup>CAZ</sup>	3.76±0.39 <sup>CAZ</sup>	3.43±0.29 <sup>CAZ</sup>
	14	3.91±0.47 <sup>aBAX</sup>	4.45±0.56 <sup>aAX</sup>	4.12±0.48 <sup>aBAX</sup>	4.30±0.28 <sup>aAX</sup>	4.31±0.30 <sup>aAX</sup>	4.64±0.58 <sup>aAX</sup>	4.02±0.15 <sup>aAX</sup>	3.76±0.54 <sup>aAX</sup>	3.45±0.38 <sup>aAX</sup>
	28	4.11±0.18 <sup>aBAX</sup>	4.27±0.20 <sup>aBAX</sup>	3.82±0.31 <sup>aAX</sup>	4.36±0.43 <sup>aAX</sup>	4.51±0.24 <sup>aAX</sup>	4.18±0.34 <sup>aAX</sup>	4.16±0.65 <sup>aAX</sup>	4.07±0.28 <sup>aAX</sup>	3.78±0.14 <sup>aAX</sup>
$b^*$	1	7.43±1.23 <sup>aAX</sup>	6.96±0.26 <sup>aAX</sup>	7.28±0.70 <sup>aAX</sup>	10.21±0.82 <sup>BAZ</sup>	10.60±0.34 <sup>BAZ</sup>	10.84±0.60 <sup>BAZ</sup>	11.17±0.68 <sup>CAZ</sup>	11.35±0.46 <sup>CAZ</sup>	10.98±0.38 <sup>CAZ</sup>
	14	7.48±0.67 <sup>aAX</sup>	5.99±0.29 <sup>aAX</sup>	7.60±0.74 <sup>aAX</sup>	8.83±0.60 <sup>BAZ</sup>	9.45±0.92 <sup>BAZ</sup>	10.00±1.47 <sup>BAZ</sup>	10.91±0.87 <sup>CAZ</sup>	11.69±0.86 <sup>CAZ</sup>	11.68±0.59 <sup>CAZ</sup>
	28	7.01±0.68 <sup>aAX</sup>	6.44±1.04 <sup>aAX</sup>	7.11±0.54 <sup>aAX</sup>	8.90±0.47 <sup>BAZ</sup>	9.66±0.52 <sup>BAZ</sup>	8.98±0.58 <sup>BAZ</sup>	11.18±1.32 <sup>CAZ</sup>	10.98±0.53 <sup>CAZ</sup>	11.17±0.41 <sup>CAZ</sup>
WHC %	1	1.63±0.25 <sup>aAX</sup>	1.66±0.05 <sup>aAX</sup>	1.55±0.21 <sup>aAX</sup>	2.00±0.02 <sup>aAX</sup>	1.98±0.60 <sup>aAX</sup>	1.88±0.26 <sup>aAX</sup>	1.96±0.14 <sup>aAX</sup>	1.67±0.41 <sup>aAX</sup>	1.96±0.15 <sup>aAX</sup>
	14	1.28±0.02 <sup>aAX</sup>	1.46±0.24 <sup>aAX</sup>	1.23±0.06 <sup>aAX</sup>	1.82±0.10 <sup>aAX</sup>	1.85±0.21 <sup>aAX</sup>	1.84±0.04 <sup>aAX</sup>	2.24±0.18 <sup>aBZ</sup>	1.70±0.06 <sup>aAX</sup>	1.82±0.04 <sup>aBZ</sup>
	28	1.76±0.42 <sup>aAX</sup>	1.39±0.08 <sup>aAX</sup>	1.31±0.16 <sup>aAX</sup>	2.00±0.02 <sup>aAX</sup>	1.97±0.23 <sup>aAX</sup>	1.99±0.01 <sup>aAX</sup>	2.17±0.18 <sup>aAX</sup>	2.03±0.53 <sup>aAX</sup>	1.91±0.07 <sup>aAX</sup>

<sup>a, b, c</sup>Means in the same row with various superscripts differ significantly regarding the difference in HHP. <sup>A, B, C</sup>Means in the same row with various superscripts differ significantly regarding the concentrations of CARV. and <sup>X, Y, Z</sup>Means in the same column with various superscripts differ significantly regarding the time of storage ( $P < 0.05$ ).

in  $L^*$  was noticed toward the end of the storage time in meat containing CARV, control, and HHP 300 MPa while a reduction rate was observed in meat pressurized with 600 MPa. The  $L^*$  values in food colour, particularly in meat products, are related to several factors, such as type and the concentrations of existing pigments, WHC, the hygroscopicity of the material that dissolves in water matrix, concentration and types of the BACs applied (Viuda-Martos et al. 2010). The meat's paleness properties may result from a rise in the  $L^*$  value that might be caused by an increase in oxidation that leads to the development of rancidity characteristics and affects the acceptability of consumers to the meat (Karabagias et al. 2011). However, CARV only with HHP showed the potential activity toward the stability of  $L^*$  value in meat compared to untreated meat. The current result was confirmed in our recent study by adding CARV to fresh minced chicken meat during refrigerated storage (Hussein et al. 2019). The decrease or stability of  $L^*$  value in pressurized meat with 600 MPa contained CARV may perhaps be elucidated by means of the hygroscopic materials with an intensified retaining of water; also, due to the capacity of CARV to absorb water that is available freely in the product, thus controlled  $L^*$  values (Fernández-López, 2005).

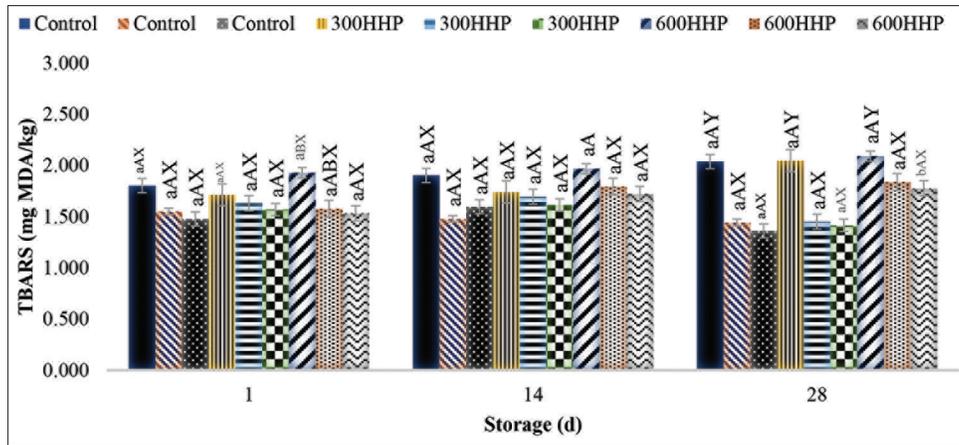
The  $a^*$  values were reduced when the level of pressure increased, and significant reductions were noticed in HHP 600 MPa compared to the control with/without CARV. Whereas no significant effect of different levels of CARV was observed in HHP-treated meat and control. Similarly, no significant difference was observed at days 1 and 28 of storage in meat treated with HHP, CARV, and control. HHP 300 and 600 MPa was very effective to increase the  $b^*$  value of the meat compared to control regardless of the contents of CARV. Additionally, various concentrations of CARV showed no significant difference compared to the control regardless of the levels of HHP. Regarding the time of storage, comparable to  $a^*$  values, insignificant variation was perceived in  $b^*$  in samples treated by HHP, CARV, and control. The results of  $a^*$  values in the current study were observed as not consistent with an increase in lightness and a decrease in yellowness. This might be due to the myoglobin that gradually oxidized and build-up metmyoglobin eventually leading to the development of discoloration characteristics of meat and meat products. Concurrently, no significant difference in  $a^*$  values was witnessed in treated samples and has a crucial contribution towards the final intensity of the meat colour, indicating the activity of CARV in stabilizing the colour of the meat. Kruk et al. (2011) treated chicken breast-fillets with 300-600 MPa HHP and in accordance to the current study they noticed that 600 MPa showed discoloration by altering colour with increasing  $L^*$ ,  $a^*$ , and  $b^*$  values while 450 MPa induced lipid oxidation. Generally, studies

have reported that HHP alters the colour values i.e., rise in  $L^*$  values and a reduction in  $a^*$  values particularly in fresh and cured meat products, resulting from dramatic changes in proteins especially sarcoplasmic myoglobin both in their conformation and integrity (De Oliveira et al., 2015).

The results from WHC are shown in (Table 1). Different levels of HHP 300 and 600 MPa significantly decreased the WHC compared to untreated meat regardless of the CARV contents. Simultaneously, no significant difference was noticed regarding various concentrations of CARV. However, at day 28 of storage stability in WHC was detected with no significant differences, particularly in samples subjected to 300 MPa HHP. The changes in pH of meat can influence the WHC and quality of meat, therefore reduction in meat pH values may cause a decrease in WHC of muscle-proteins (Shirzadegan and Falahpour, 2014). Besides, an increased pH value may deteriorate meat quickly and shorten its shelf life (Chan et al., 2011).

### TBARS

In the current study, the degree of lipid oxidation was higher in the meat treated with different levels HHP and a significantly increased in meat treated samples with 600 MPa compared to 300 MPa and control (Fig. 1). With respect to the content of CARV a significant difference was noticed between samples treated with 1000 ppm compared to meat containing 500 ppm of CARV and control, the differences were much more pronounced at day 28 of storage. During the storage period, reductions in TBARS were found in control-CARV 500ppm, control CARV 1000ppm, 300HHP-CARV 500ppm, and 300HHP-CARV 1000ppm. However, a significant increase only was observed in the meat treated with HHP that do not contain CARV i.e., control No-CARV, samples of 300HHP-No-CARV and 600HHP-No-CARV ( $P < 0.05$ ). In accordance with our study De Oliveira et al. (2015) noticed an increased TBARS index towards the end of storage and the pressurized samples exhibited TBARS scores of 0.108 - 0.143 mg MDA/kg. Moreover, Kruk et al. (2011) noticed that treatment with HHP (approximately 450 MPa) induces lipid oxidation in chicken breast fillets, and TBAR values almost doubled at 600 MPa for 7 days of storage. It has been stated in several studies that high pressure can trigger the process of lipid oxidation in meat and meat products while the complete mechanisms is unknown by which chemical reactions alter and induces lipid oxidation by HHP through the thermodynamic equilibrium. The general hypothesis for this mechanism, however, is that membrane rupture and haemoproteins cause an increase in the release and accessibility of iron, and that this release can facilitate the process of lipid oxidation (Medina- Meza et al., 2014; Bajovic et al., 2012). Besides, autoxidation is recognized as a main pathway in the oxidative degradation

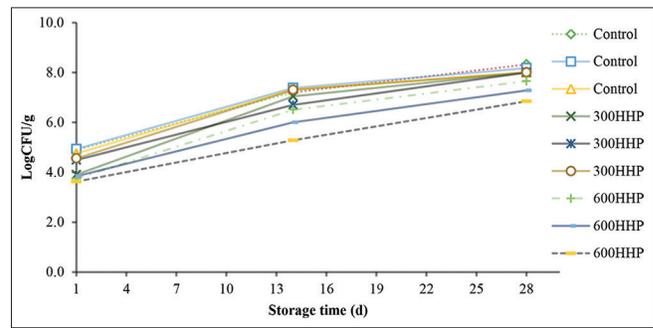


**Fig 1.** Effect of various concentrations of CARV and different levels of HHP on TBARS of fresh chicken meat kept at 4°C. <sup>abc</sup>Means with various superscripts differ significantly regarding the difference in HHP. <sup>A,B,C</sup>Means with various superscripts differ significantly regarding the concentrations of CARV ( $P < 0.05$ ). <sup>xyZ</sup>Means with various superscripts differ significantly regarding the time of storage.

of fat in foods, particularly in the hydrolytic, enzymatic, and photooxidation mechanisms (Bajovic et al., 2012). It has been stated that TBA  $\geq 5$  mg MDA/kg meat comprises the threshold for detecting off-flavour for humans (Karabagias et al., 2011). In the current investigation, such a high amount of lipid oxidation was not witnessed. The addition of phenolic BACs as natural antioxidants such as CARV can decrease the formation and absorption of MDA and promote removing or inactivating of free radicals in systems formed throughout the reactions (initiation or propagation), by giving atoms of hydrogen to these molecules and preventing chain reactions. The aromatic ring promotes resonance, which stabilizes the structure of the molecule (Falowo et al., 2014; De Oliveira et al., 2015).

**Microbiological characteristics**

The antimicrobial activity of CARV and HHP against AMC of chicken meat are presented in Fig. 2. Regarding the various concentration of CARV and levels of HHP reduction in the AMC population were noticed at 14 of storage in 600HHP-CARV 1000 ppm about 1.9, 2.1, and 1.7 logs compared control No-CARV, control CARV-500 ppm, and control CARV-1000 ppm, respectively, which was the average of 1.2 logCFU/g reduction in sample HHP 600HHP-CARV 1000 ppm, while it was about 0.3 log reduction at day 28. At day 28 similar increase in the AMCs was observed in all meat samples. However, reductions about 0.6, 0.8, and 1.1 log in AMCs were seen in meat treated with 600HHP-No CARV, HHP300-CARV 500 ppm, and 600HHP-CARV 1000 ppm compared to control-No CARV, control-CARV 500 ppm and control-CARV 1000 ppm, respectively. In a study by Duranton et al. (2012) they found that using hurdle-like salt combined with HHP exhibited a better effect in pork meat compared to applying salt or HHP alone. They hypothesized that the application of the hurdle could result in a synergy between 350 MPa and salt due to bacterial cells’ increased sensitivity to

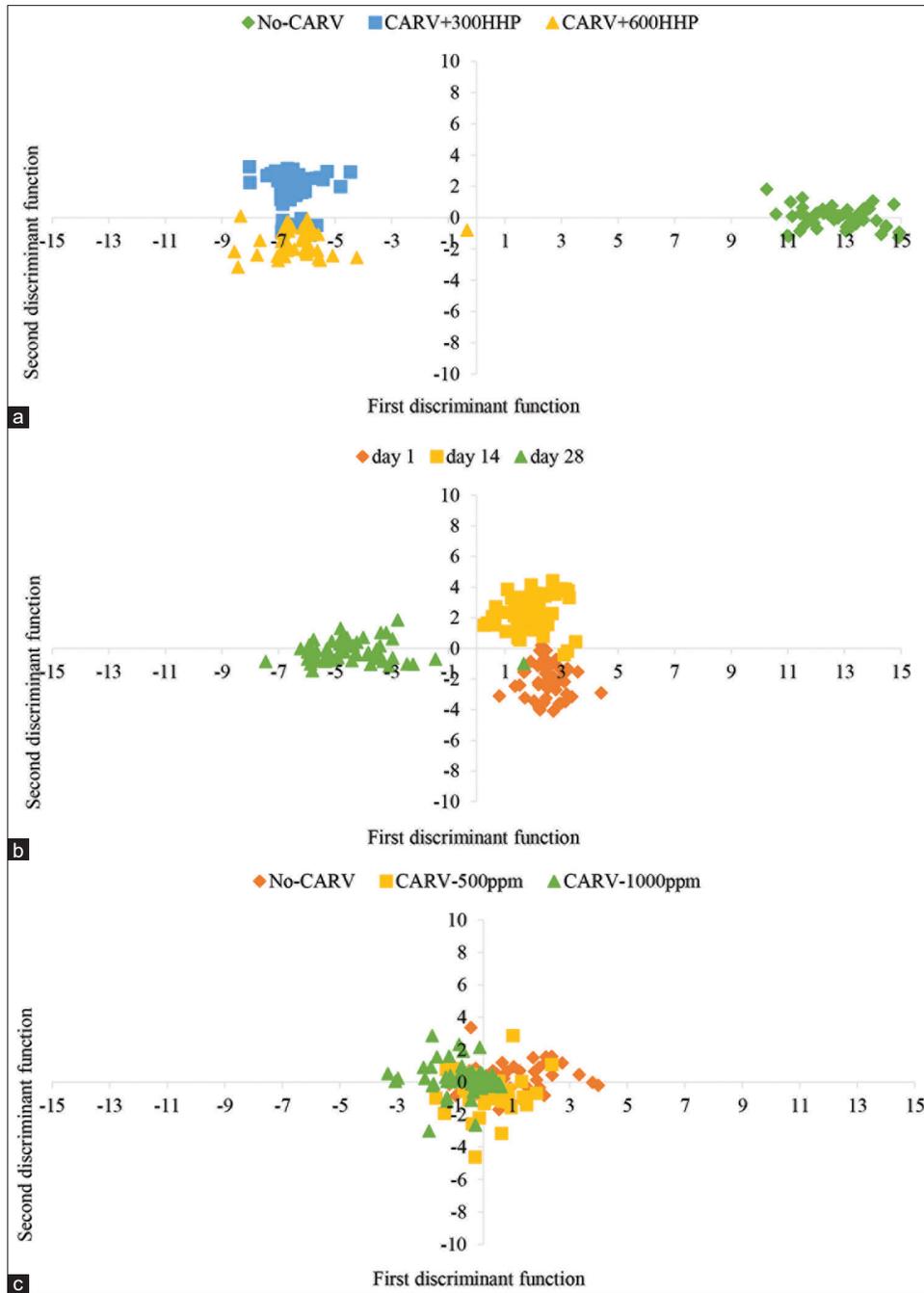


**Fig 2.** Shows the impact of various CARV concentrations and HHP levels on the aerobic mesophilic counts of chicken meat kept for 28 days at 4 °C.

pressure, and that the combined treatment led to low microbial counts (below 2 logCFU/g) at the end of storing period. The result from the present study indicates that the meat treated with HHP600 No-CARV and HHP600 CARV-500 ppm is considered microbiologically safe and acceptable until 21 d of storage, while it could reach 28 days for the sample treated with HHP600 CARV-1000 ppm, because of not exceeding the maximum microbial limits in bacteria populations for acceptable quality meat which is about 7 log logCFU/g (Karabagias et al., 2011). According to Ultee et al. (2002), CARV disrupts the physical structure of the fatty acid chains by interacting with the cell membrane and dissolving the phospholipid bilayer. Consequently, the fluidity of the cell membranes rises due to the expansion and instability, which then makes passive permeability more prevalent.

**Electronic nose**

In the present study, the aroma pattern of fresh chicken meat subjected to different levels of HHP changed during refrigerated storage for 28 days under vacuum (Fig. 3). Using CDA, the ability of the E-nose to distinguish between untreated meat and the groups supplemented with HHP and CARV was clear. The aroma pattern of



**Fig 3.** Shows the effectiveness of various CARV concentrations and different levels of HHP on E-nose smell detection in chicken meat refrigerated at 4 °C. Along with a score plot from a canonical discriminant analysis of the separation based on the various: (a) HHP levels, (b) storage time, and (c) concentrations of BACs

untreated samples was always discriminated from samples subjected to HHP during storage. Different levels of HHP were remained toward the 2<sup>nd</sup> discriminant function while control meat was detected at the 1<sup>st</sup> discriminant function (Fig. 3a). Regarding the storage period the same direction of aroma detection were observed on day 1 and 14, while on day 28 opposite direction toward 2<sup>nd</sup> discriminant function with intensifying odour were noticed (Fig. 3b). Furthermore, overlapping between meat that contained

CARV and control groups were noticed, whereas a clear tendency toward 2<sup>nd</sup> discriminant function was observed for meat treated with CARV (Fig. 3c). Alongside, reduced lipid oxidation was noticed with CARV, simultaneously significant increase in TBARS only was observed in the meat treated with HHP that do not contain CARV ( $P < 0.05$ ). This indicates that CARV exhibited no changes in perception of off-odour throughout the period of storage and the E-nose can categorize the chicken flesh as

either fresh or spoiled due to the intense flavour rancidity. Previously, it has been investigated that the response of E-nose device in the meat industry is not only narrowed in the classification of meat and detection of sensory attributes (off-flavours), but also with total biogenic amine content, microbiological status, and estimation of shelf life (Ramírez et al., 2017). It is known that the most important attributes for the consumer during meat consumption are juiciness, tenderness, flavour, and the absence of off-flavours (Nowicka et al., 2017). In this study, it was noted that CARV produced a spicy odour that was noticed plentifully upon opening the bags and could create appealing flavour characteristics for particular foods, such as meat, and boost customer acceptance (Hussein et al., 2019). Additionally, numerous other factors can be taken into point during the application of E-nose in meat, such as the fat content, advancement of lipid oxidation, the release of fatty acids, and increased microbial load during storage. These factors may promote modifications to the profile of meat's aroma throughout the period of storage (Mildner-Szkudlarz et al., 2007; Djenane et al., 2003).

## CONCLUSION

In the present study, the changes in physicochemical attributes, lipid oxidation, odour changes (e-nose odour-based), and the response of AMCs to HHP combined with different concentrations of carvacrol are addressed. CARV maintained the stability of meat pH during refrigerated storage in samples treated with/without HHP compared to the control. At the end of the storage an increase in lightness, decrease in redness, and decreased WHC was observed in HHP-treated meat irrespective of the concentration of CARV. Moreover, at day 28, reductions in TBARS were found in meat treated with various concentrations of CARV combined with a low level of HHP, while HHP 600 MPa significantly increased lipid oxidation. The current results indicate that the meat treated with HHP600 CARV-500 ppm is considered microbiologically safe until 21 d of storage and it could reach 28 days for samples treated with HHP600 CARV-1000 ppm. Furthermore, the aroma pattern of control was discriminated from samples subjected to HHP during storage. Accordingly, the addition of CARV enhanced the preservation effects of HHP on fresh vacuum-packed chicken meat. More studies are required to fully comprehend the physicochemical and morphological changes that pressurized meat undergoes, as well as in what manner these variations influence the qualitative characteristics of chicken meat.

## Conflicts of interest

There is no conflict of interest to be declared by the authors.

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## Authors contributions

This manuscript contains the results from the work carried out collaboratively as below: KNH: investigation, visualization, conceptualization; TC: funding acquisition; BM and HA: review and editing; AVT, KH, JG, and CN: methodology and resources, Laboratory HHP treatment; LF and ID supervising the work, data analysis. All authors agreed on this manuscript version for publication.

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