



## Effect of the Cold Plasma and Aqueous Grape Seeds Extract on Minced Beef

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### Abstract

Cold plasma (Cp) technology has developed in recent decades as a method of processing meat that functions as a bacteriostatic and bactericide, and it is used to treat meat as a non-thermal technique to preserve the sensory qualities of meat. Yet, one disadvantage of this approach is lipid oxidation. The purpose of this research is to investigate the effect of aqueous grape seed extract at (1%) concentration after spraying it onto meat (100 ml/kg) and then treating it with cold plasma (plasma jet) using argon gas at a frequency of 23 kHz, a voltage of 150 kV, a distance of 2 cm, and a time of 5 minutes, as well as to demonstrate the extent of its effect on the pH, total bacterial count (TBC), thiobarbituric acid substance (TBARs), peroxide value (PV) of meat at 4 °C for 12 days. We separated 100 samples of minced beef into three groups. Group (1)- Control (Con), group (2)-Cold plasma (Cp), group (3)-Grape seeds extract (GSE), and group (4)-Cold plasma mixed Grape seed extract (CP+GSE): Each group has 25 samples, and all parameters were measured. The results showed that grape seed extract and cold plasma (Cp+GSE) work well together (a synergistic relationship), and it was the highest group that reduced the number of bacteria and the lipid oxidation level for 12 days at a temperature of 4 °C.

**Keywords:** Cold Plasma, Aqueous Grape Seeds Extract, Contaminated Minced Beef

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### 1.Introduction

Beef is favored by people due to its high protein content, various vital amino acids, vitamins, and low fat content. On the other hand, beef is prone to microbiological contamination and chemical degradation during transportation, storage, and distribution, which may risk human health (Muchenje et al., 2009). Because of its high nutritional content and high water activity, ground beef is susceptible to bacterial deterioration (Salum, 2010; Ercolini et al., 2011). As a consequence, microbial proliferation and increased lipid peroxidation in refrigerated minced beef result in decreased nutritional value and shorter meat product storage life (Dave & Ghaly, 2011). Additionally, people interested in a healthy life require more fresh or unprocessed meals. Foodborne illness may result from microbiological issues. As a result, new sterilizing technologies are needed. Non-thermal procedures retain odor and taste while enhancing food safety without losing quality. Alternative food processing methods are becoming popular because of these benefits. One of these new approaches is plasma (Al-Azawi et al., 2019; C. M.G. Charoux et al., 2021; Clémentine M.G. Charoux et al., 2020; Lee et al., 2017; Mir et al., 2020).

The use of cold plasma as a technique in the food industry is a relatively new development. It's cheap and environmental, therefore it's getting popular (Pankaj et al., 2018; Shihab et

**al., 2021**). Plasma is usually referred to as the fourth state of matter, according to a system illustrating an increase in the degree of energy from solid to liquid to gas, and finally to an ionized state of the gas plasma (**Fridman et al., 2008**). At atmospheric pressure, the most common working gases used to make non-thermal plasmas are noble gases, such as Argon (Ar) (**Moravej et al., 2004; Li et al., 2006; Shihab., 2018**). Otherwise, (**Nehra et al., 2008**) say that the plasma jet (PJ) makes a discharge that is steady, consistent, and uniform even when it is running at atmospheric pressure. **Misra et al, (2011)** who conducted one of the first studies to investigate how cold plasma may be used to keep food safe, highlighted the importance of investigating how plasma impacted what occurred to lipids.

In addition to the advantages of natural ones, (**Bañón et al., 2007; Ganhão et al., 2011; Dakheel et al., 2022**) show that phenolic compounds are found in a lot of foods, especially fruits and vegetables. These compounds are known to have antimicrobial and antioxidant properties. Moreover, microbial degradation and oxidative reactions shorten the shelf life of meat. People are looking for better alternatives to synthetic food preservatives due to health concerns. Natural preservatives are becoming more popular among food makers in order to meet consumer demand (**Beya et al., 2021; Jeboory, 2012**). In recent years, several plant extracts have grown in popularity as beneficial dietary components. Grape seed extract (GSE) is rich in phenolic components, including monomeric flavan-3-ols, procyanidins, and different compounds, all of which have antibacterial and antioxidative activities (**Khanal et al., 2009**). Further, some researchers, such as (**Munekata et al., 2020**) discovered that grape seed (GS) appears to be among the most adaptable sources of antioxidants due to the proper conservation and protection of fresh, cooked, fermented, and dry-cured meats. In addition, grape seeds are a great source of naturally occurring phenolic chemicals and the aqueous extract of grape seeds seems to have larger concentrations of Gallic acid and catechine, and distilled water is an effective extraction agent and favored solvent since it is nontoxic, environmental safe, and cheap (**Ignat et al., 2011**).

## 2-Materials and Methods

### 2.1 Preparation of aqueous grape seeds extract

Grape seeds were bought from a market and taken to the research facility, where they were washed with water and left to dry at room temperature. It was then dried at 40 °C for 8 hours before being ground into a fine powder with a grinder (Silver Crest, China, Item No.: OG-606). 5 g of the resulting powder was extracted and added to 50 ml of almost boiling water. It was then allowed to reach room temperature. The samples were centrifuged for 10 minutes at 16,000 rpm after being filtered via cheesecloth (**Chedea et al., 2011**). However, I modified 4 steps in this extraction to produce better results:

1. mixed 30 g of seed powder with 300 ml of water on a magnetic stirrer for 6 hours at a temperature no higher than 40 °C.
2. The mixture is first filtered through cheesecloth and then through a Whatman No. 1 paper filter.
3. The mixture should be kept in an incubator at a temperature of no more than 40 °C until it is dry.
4. After the mixture has fully dried, 1 g of it is dissolved in 100 ml of normal saline until a concentration of 1% is achieved, and mixed in vortex for 3 minutes.

The antibacterial activity of GSE was determined according to **Sağdıç et al., (2002)** and **Sağdıç & Özcan, (2003)** and we also estimated the inhibition zone diameter (mm) of GSE. Minced beef was purchased locally and transferred to the laboratory in a cool box; the meat was then separated into 4 groups, each group contain 25 samples (each sample weight 25 g ). The first group received CP treatment , the second group received GSE treatment, the third group received a mix of CP and GSE treatment, and the fourth group served as a control. Beef samples were stored in sterile polyethylene bags measuring 20 cm 15 cm (Falcon; Sharjah, United Arab Emirates NB-0002) at 4 °C for 12 days, fig(2-4). The total bacterial count (TBC), PH, peroxide value (PV), panal test, as well as On days 0, 3, 6, 9, and 12, TBARs were evaluated from the refrigerator using a random samples. The sole gas utilized in this experiment was argon, and it employed a technique known as plasma jets. The minced meat was subjected to internal voltages of 150 kV, frequencies of 23 KHz, gas flow rates of 5 h/min, plasma bundle lengths of 3 cm, and tube diameters of 0.5 cm. The sample was then placed in a petri dish 2 cm from the tube for 5 minutes. In this study, the frequency and voltage were changed to be different than in previous studies such as(Lu et al., 2012; Misra & Jo, 2017) , yet within the range that kills bacteria and prevent lipid oxidation at same time.

In this experiment, the spraying method was used. The meat was put on a clean plate inside the hood, and 100 mL/kg of GSE at a concentration of 1% was sprayed on it. PH test was estimated(**Nirmal & Benjakul, (2011)**),total bacterial count (TBC)(**Miles et al., 1938**). Thiobarbituric acid reactive substances (TBARS) test was done as mentioned by ( Buege & Aust, 1978; Al-Doski et al., 2021). The peroxide value (PV) was estimated using the procedure of **Low & Ng. ( 1978)**.

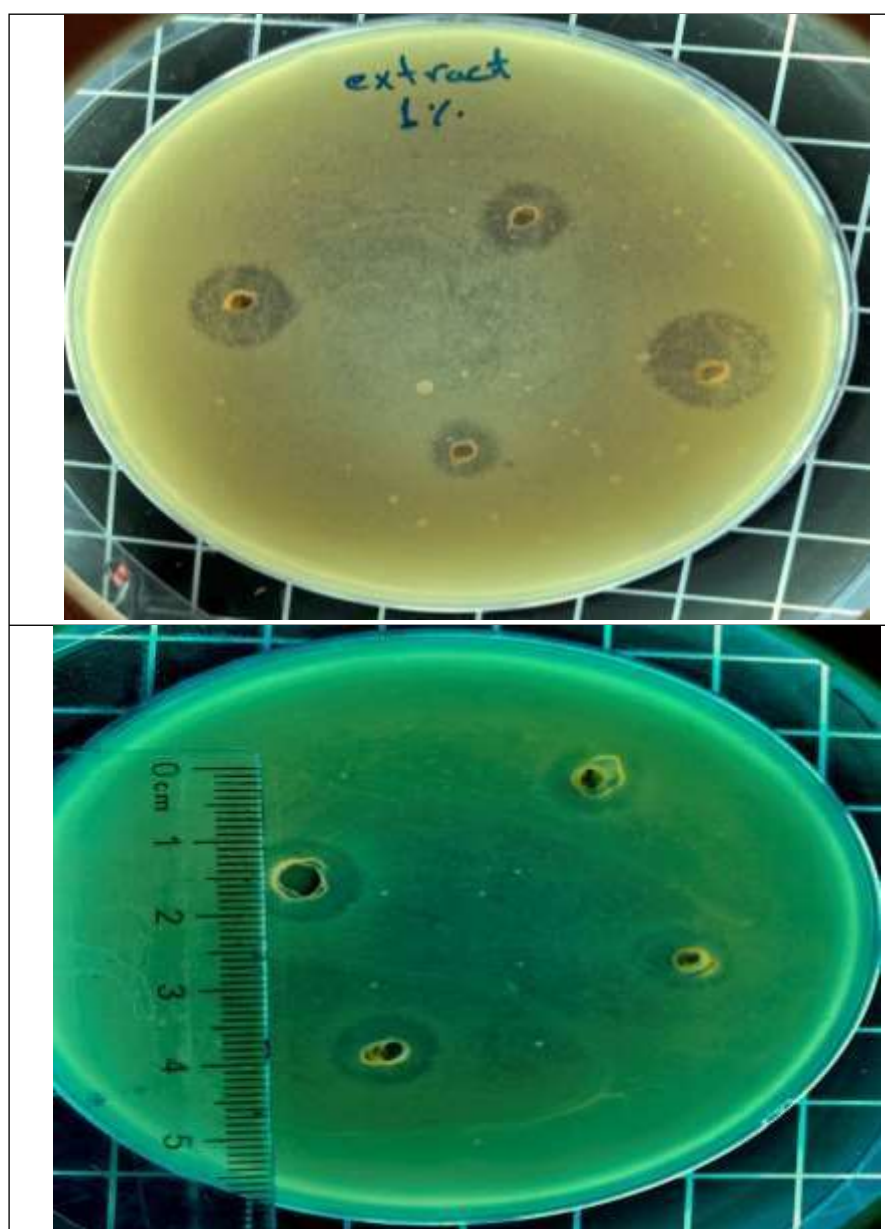
### Statistical analysis

SAS was used to do statistical analysis of the data (Statistical Analysis System - version 9.1). two-way ANOVA and Least Significant Differences (LSD) post hoc test were used to determine if there were significant differences between means. Correlation coefficients were also estimated.  $P < 0.05$  is considered statistically significant.

## 3-Results and Discussion

### 3.1 Inhibition zone diameter (mm) of GSE

(Figure 3-1) displayed the effects of antibacterial activity as determined by the width of the inhibition zone (IZ) at 0.1%, 0.5%, and 1% concentrations. At the 1% concentration, the diameters varied from 9 to 14 mm. while the remaining concentrations (0.1% and 0.5%) did not showed a result. These results are similar to results obtained by **Baydar et al., (2006)** who found At 0.5% and 1% concentrations, grape seed extracts were bacteriostatic against *E. coli O157:H7*, but bactericidal at 2.5%. All extracts were bactericidal against *S. aureus* after 48 hours and *A. hydrophila* after 1 hour. **Tesaki et al., (1999)** showed that phenolic chemicals such as gallic acid may be the source of food-borne microbial inhibition. However, grape seed extracts were shown to have antibacterial activity against 14 different microorganisms ( **Baydar et al., 2004**). Also, According to some researchers, such as **Nilgun Gokturk Baydar et al. (2006)**, all extracts had comparable effects on infections, depending on concentration and bacterial type. Total phenolics were high in grape seed extracts.



**Fig .1 Inhibition zones diameter of grape seed extracts 1%**

### **3.2 Effect of treatments and periods of storage on the P**

The results in (Table 1) and (Figure 2) illustrated the effects of treatments and periods of storage on the PH of ground meat. The effect of periods of storage for each groupe was significant ( $P<0.05$ ) between each of 0, 3, 6, and 9 days as compared with 12 days in (Cp and Cp+GSE groups). While the onther groups were significant ( $P<0.05$ ) between (0 and 3 days) as compared with 9 and 12 days in the GSE groupe and (0, 3, 6 days)as compared with 9 and 12 days in the Con groupe.

Concerning the differences among groups within periods, results showed that there was no statistically significant difference ( $P>0.05$ ) among groups within the periods of (0, 3, and 6 days), while a significant difference was detected for 9 days as the treatment groups ( Cp, GSE, and CP+GSE) showed a significant ( $P<0.05$ ) lowest pH (5.93,6.08, and 6.02

respectively) as compared with control (7.65). On 12<sup>th</sup> day, the GSE group showed lowest PH as compared with onther groups. These results are corroborated with results obtained by **Bauer et al., (2017)**, who confirmed the non-significant effect of the CP on the pH. Similar results were obtained by some researchers (**Bañón et al., 2007; Rojas & Brewer, 2007**) in regards to the non-significant effect of on pH until 9 days. Additionally, a previous study conducted by **Reham & Shimaa, (2017)** illustrated that GSE decrease the pH up to 9 days of refrigeration meat. However, the microbial activity during storage could cause protein breakdown and release of protein metabolites, most notably amines, which could lead to increase the pH during a storage (**Reddy et al., 2013**). On the other hand, these findings conflict with other researchers (Webb et al., 2005; Odhaib et al., 2021), who indicated that the pH level significantly affects tenderness, storage duration, and color of meat . However, there is no significant difference in the pH of CP group on the third, sixth, and ninth days, despite a change in color and tenderness since the start of treatment. In contrast, no change in color of the GSE group although the pH increased at 12<sup>th</sup> day.

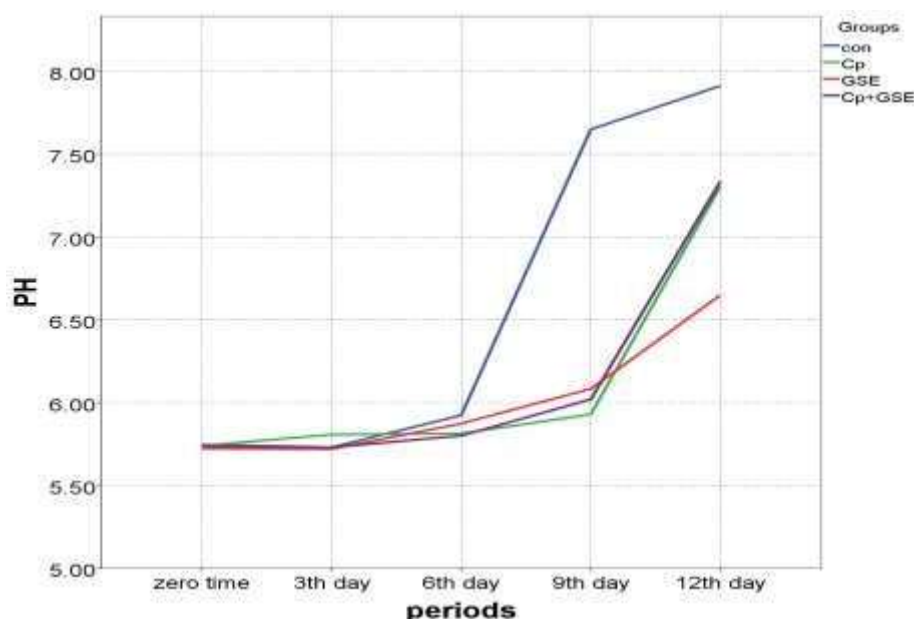
**Table 1 Effect of treatment and periods of storage on PH**

Groups. pH	Zero time	3 days	6 days	9 days	12 days
<b>Con</b>	<b>B5.74±0.01a</b>	<b>B5.73±0.01a</b>	<b>B5.92±0.08a</b>	<b>A7.65±0.05a</b>	<b>A7.91±0.03a</b>
<b>CP</b>	<b>B5.74±0.01a</b>	<b>B5.80±0.01a</b>	<b>B5.81±0.02a</b>	<b>B5.93±0.04b</b>	<b>A7.30±0.12b</b>
<b>GSE</b>	<b>C5.74±0.01a</b>	<b>C5.72±0.00a</b>	<b>BC5.87±0.03a</b>	<b>B6.08±0.18b</b>	<b>A6.65±0.20c</b>
<b>CP+GSE</b>	<b>B5.74±0.01a</b>	<b>B5.73±0.00a</b>	<b>B5.80±0.01a</b>	<b>B6.02±0.21b</b>	<b>A7.33±0.18b</b>
<b>LSD</b>	<b>0.30</b>				

Means with a different small letter in the same column are significantly different (P<0.05).

Means with a different capital letter in the same row are significantly different (P<0.05).

Mean ±Standard Error. N = 3, Con = control, Cp = Cold plasma, GSE = Grape seeds extract.



**Fig 2 Effect of treatment and periods of storage on the PH**

### 3.3 Effect of treatments and periods of storage on the total bacterial count (TBC)

The results indicated that the differences among periods of the storage were significant ( $P < 0.05$ ) for all groups (Table 2) (Figure 3). The trend of change in all groups tends to decrease significantly ( $P < 0.05$ ) along with advanced storage periods (0, 3, and 6 days) and then significantly ( $P < 0.05$ ) rise again in the (9 and 12 days). These results indicated that the cooling caused an inhibition with a varied degree among all groups.

Concerning the differences among groups within periods, the findings revealed a significant difference ( $P < 0.05$ ) between groups during the periods of (3, 6, 9 and 12 days), where the CP+GSE group was recorded a higher reduction in TBC at day (3<sup>rd</sup>, 6<sup>th</sup>, and 9<sup>th</sup>), while the GSE group showed a higher drop at 12<sup>th</sup> day.

The reduction in the TBC could be attributed to the action of chilling on bacteria in addition to the effect of treatments, which cause an inhibition of growth to varying degrees. Previous research also reported the decline of the TBC over the storage period (Shrestha et al., 2012; Zimoch & Jarmoluk, 2012). They highlighted this drop in TBC as a result of GSE and CP influence on the meat. These findings are comparable to those of Moore & Gill, (1987), Kim et al., (2011), Bellés et al., (2017), who suggest that bacterial activity may enhanced by an increase in the pH caused by refrigerated storage. However, these findings suggest that bacterial cells are unable to fully recover after storage post-plasma treatments. It should be noted that the effectiveness of plasma in killing bacterial cells continues after

Table 2 Effect of treatment and periods of storage on total bacterial count (TBC)

Groups TBC Log <sub>10</sub> CFU/g	Zero time	3 days	6 days	9 days	12 days
Control	A9.36±0.01c	B8.36±0.01d	C8.15±0.03c	D9.59±0.10d	AB8.87±0.02d
Cold plasma	A9.25±0.04b	B8.02±0.10c	C7.54±0.06b	D9.14±0.13c	C8.32±0.13c
GSE	A9.23±0.03ab	B7.71±0.16b	C7.55±0.07b	D8.97±0.15b	B7.77±0.07a
CP+GSE	A9.13±0.07a	C6.24±0.03a	D6.09±0.05a	B7.98±0.07a	B7.99±0.06b
LSD	0.10				

Means with a different small letter in the same column are significantly different (P<0.05).

Means with a different capital letter in the same row are significantly different (P<0.05).

Mean ±Standard Error. N = 3, Con = control, Cp = Cold plasma, GSE = Grape seeds extract.

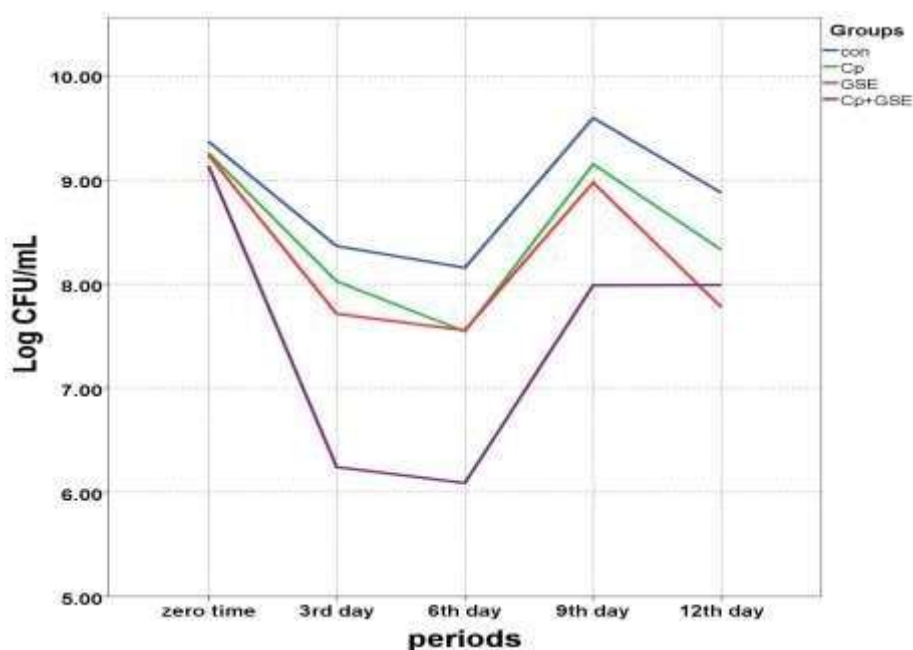


Fig 3 Effect of treatments and periods of storage on the total bacterial count (TBC)



treatment, and the effects during this period are also influenced by the storage conditions (Shen et al., 2016).

### 3.4 Effect of treatments and periods of storage on the on Thiobarbituric acid-reactive substances (TBARs)

The rate of change of the TBARs varied significantly ( $P < 0.05$ ) across periods but with different trends and the appearance of the pink color is an indication of the amount of secondary lipid oxidation (Figure 5). The means of the TBARs fluctuated between raising and reduction. The highest mean (2.39) was detected on 9 days (Table 3) (Figure 4) then decreased significantly on 12 days.

The CP group showed non-significant differences during the periods 0, 3, and 6 days then showed a significant increasing ( $P < 0.05$ ) on 9, and 12 days as compared with previous periods. Similar rate of change was shown in the GSE group as the differences during the periods 0, 3, and 6 days were not significant. Then the means increased significantly ( $P < 0.05$ ) on 9, and 12 days. The rate of change in the CP+GSE group is identical to that of the CP. Concerning the differences among groups within each period, there were no significant differences on 0, 3, 6 days, whereas the differences were significant on 9 days. The mean of the control (2.39) was significantly higher than other groups, followed by the CP (1.82), and the lowest in the GSE (1.40).

This result is consistent with a prior study of **Guzmán-Chozas et al. (1998)**, who found that the TBARs test is a commonly used method for measuring lipid oxidation by detecting the concentration of a secondary degradation product, namely malonaldehyde. When heated inside an acidic solution, malonaldehyde and TBA mix to form a pink Schiff base combination with a maximum absorbance between 532 and 534 nm (Figure 7). On the other hand, this result supports a prior study by **Brannan & Mah, (2007)** who reported that both raw and cooked meat may benefit from GSE's antioxidant properties. Polyethylene bags may have a minor effect on lipid oxidation. Similarly, **Rubio et al. (2008)** mentioned that the packaging strategies utilized during refrigerated storage had a minor effect on the stability of lipid oxidation. In contrast, according to the association between PV and TBARS, the results of this study disagree the findings of previous researchers, such as (**Zhang et al., 2016**) that a drop in PV led to a decrease in TBARS values when the correlation between PV and TBARS was examined.

**Table 3 Effect of treatments and periods of storage on the on Thiobarbituric acid-reactive substances (TBARs)**

Groups	Zero time	3 days	6 days	9 days	12 days
Control	D0.67±0.03a	C1.00±0.02a	CD0.87±0.07a	A2.39±0.06c	B2.01±0.30b
CP	B0.95±0.02a	B0.99±0.06a	B0.90±0.11a	A1.82±0.10b	A1.98±0.16b



<b>GSE</b>	<b>C0.98±0.04a</b>	<b>C0.90±0.05a</b>	<b>C0.84±0.07a</b>	<b>B1.40±0.02a</b>	<b>A1.83±0.16ab</b>
<b>CP+GSE</b>	<b>B0.81±0.04a</b>	<b>B0.88±0.03a</b>	<b>B0.72±0.05a</b>	<b>A1.59±0.05ab</b>	<b>A1.66±0.16a</b>
<b>LSD</b>	<b>0.30</b>				

Means with a different small letter in the same column are significantly different ( $P < 0.05$ ).

Means with a different capital letter in the same row are significantly different ( $P < 0.05$ ).

Mean  $\pm$  Standard Error. N = 3, Con = control, Cp = Cold plasma, GSE = Grape seeds extract.

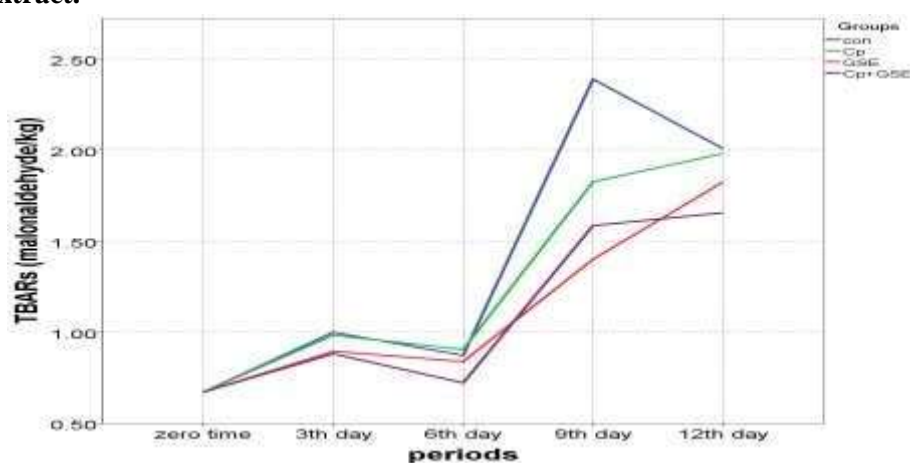


Fig 4 Effect of treatments and periods of storage on the on Thiobarbituric acid-reactive substances (TBARS)



Fig 5 The reaction of malonaldehyde with thiobarbituric acid (TBA) results in the formation of a pink Schiff base.

### 3.5 Effect of treatments and periods of storage on peroxide value (PV)

The findings in (Table 4) and (Figure 6) showed that there were no statistically significant differences in PV among the three groups and the control group at zero time. On day three there were significant differences among the groups ( $p < 0.05$ ). (CP + GSE) and CP showed the highest means, followed by GSE, and lastly, control. This rise was induced by the quick synthesis of hydroperoxide during the first phase. On day six, no significant differences were shown. However, as compared to the prior period, hydroperoxide formation reduced in all groups. while on day nine, significant differences were observed ( $p < 0.05$ ) between treatment and control groups, (CP + GSE) group showed highest scoring, followed by the GSE and CP groups, respectively, and finally the control group. On day 12, significant statistical differences were found ( $P < 0.05$ ), as the GSE group exhibiting the lowest peroxide generation, followed by the CP + GSE, the control, and lastly, the CP (Table 4). Hydroperoxides are the principal products of peroxidation and are responsible for determining the degree of lipid oxidation during the early phases of oxidation (Juntachote *et al.*, 2006). The PV increased with storage time in the current study because of the synthesis of new hydroperoxides was faster than their breakdown into secondary products. In contrast, whenever the degradation of hydroperoxides rose faster than the production of new hydroperoxides, the PV decreased. Similar findings of increasing and then decreasing PV throughout the course of storage time have been recorded by Teets and Were., (2008). During refrigerated storage, samples containing GSE produced much less peroxide than samples containing CP ( $P < 0.05$ ), most likely because their polyphenolic and flavonoid contents serve as antioxidants, ending free radical chain-type processes (Juntachote *et al.*, 2006). Additionally, this finding corroborated the findings of Saint-Cricq de Gaulejac, (1999) who established that antioxidants may protect cells from free radical damage.

**Table 4 Effect of treatments and periods of storage on peroxide value (PV)**

Groups	Zero time	3 days	6 days	9 days	12 days
Con	C1.70±0.33a	A3.83±0.16b	AB3.00±0.29a	BC2.00±0.29a	BC2.33±0.17ab
Cp	C1.67±0.67a	A5.67±0.33a	BC2.33±0.33a	B2.75±0.43ab	BC2.50±0.29b
GSE	BC1.70±0.00a	A4.67±0.33a b	BC2.00±0.58a	B2.50±0.29ab	C1.33±0.33a
CP + GSE	C1.69±0.58a	A5.33±0.66a	BC2.50±0.29a	B3.50±0.29b	C2.00±0.00ab
LSD	1.01				

Means with a different small letter in the same column are significantly different ( $P < 0.05$ ).

Means with a different capital letter in the same row are significantly different ( $P < 0.05$ ).

Mean  $\pm$  Standard Error. N = 3, Con = control, Cp = Cold plasma, GSE = Grape seeds extract.

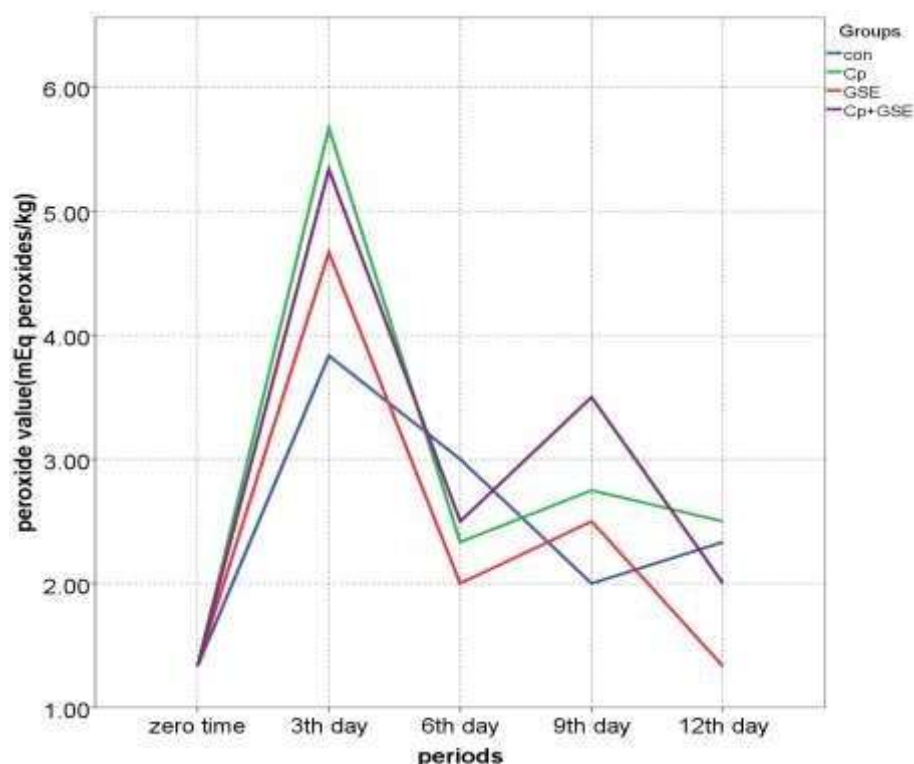


Fig 6 Effect of treatments and periods of storage on peroxide value (PV)

## Conclusions

The dose applied in the combination (Cp+GSE) showed excellent efficacy, maintaining the TBARs in the permissible limits of less than 2 Malonaldehyde with a high bacterial decrease compared to the other treatments until 12th day.

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