

## EVALUATION OF ANISE SEEDS EXTRACTS AS A NATURAL ANTIOXIDANT IN RAW CHICKEN MEAT PATTIES

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### Abstract

The efficiency of anise extracts prepared by different means in raw meat during 7 days was determined. This experiment was designed as a factorial design consisting of a  $4 \times 3$  with four treatments (water extract 100%, ethanol 100%, aqueous ethanol (ethanol: water at 70:30 v/v) and control), and three storage periods (0, 3 and 7 days). Hence, the TBARS value and free fatty acids were increased markedly in both treated and non-treated samples over 7 days. While inhibition of lipid oxidation was higher in samples contained extracts at each point of the storage period compared to the control. Moreover, the lowest TBARS value observed in chicken meat samples were treated with aqueous ethanol compared to the control. Moreover, the amount of free fatty acids was lower in all treated samples over storage time, while, the lowest value of free fatty acids was recorded in both meat samples that were treated with anise extracted by ethanol and aqueous ethanol compared to the control and water extracts respectively. Moreover, supplemented meat with anise extracts reduced drip losing ratio at each point of the storage period compared to the control. Whilst, differences were not significant ( $P > 0.05$ ) in cooking loss of meat from the four treatments. The cooking loss of meat was significantly affected by treatments ( $p < 0.001$ ). Hence, the highest amount of cooking loss was observed in control samples compared to the treated samples. Moreover, Non-significant differences were detected between the four treatments for all sensory attributes. Based on these results, the anise extracts may have the capability to retard the autoxidation of lipids and improve the quality of meat.

**Keywords:** Chicken Meat; Anise Extraction, Lipid Oxidation; Antioxidant; Free Fatty Acids

### INTRODUCTION:

Meat is a lipid-rich food containing high levels of fat and high amounts of polyunsaturated fatty acids, which are considered to have health benefits (Hayes, 2000). However, these polyunsaturated fatty acids are prone to post-slaughter oxidation due to a chemical reaction between the oxygen and lipid content (Richards et al., 2002). This auto-oxidation of lipids is the main factor that negatively affects the chemo-physical

characteristics, nutritional value, and sensory attributes of meat (Byrne et al., 2001; Min et al., 2008). Hence, the autoxidation of polyunsaturated fatty acids is the main reason of production oxidation by-products such as volatiles, hydroperoxides, malondialdehyde, and 4-hydroxynonenal which causes discoloration, unpleasant odors, and flavors of meat (Erickson, 2000; Hayes, 2008). This development of abnormal off-odors and color in meat is due to an attack by free radicals on both lipids and proteins (Nanke et al., 1998; Erickson, 2000; Ahn et al., 2001).

Lipid oxidation can be retarded by using antioxidants, through the scavenging free-radical (Erickson, 2002; Naveena et al, 2008; Velasco and Williams, 2011). Hence, antioxidant compounds are often added to fresh and pre-cooked meat to inhibit lipid oxidation and increase shelf-life (Padmashree et al, 2007; Velasco and Williams, 2011). Synthetic antioxidants such as ButylatedHydroxy Anisole (BHA) and ButylatedHydroxy Toluene (BHT) have been widely applied to several food industries to inhibit lipid oxidation (Erickson, 2002; Naveena et al, 2008; Velasco and Williams, 2011). More recently, natural antioxidants have been used as a food additive to retard the oxidative process, improve quality and increase the nutritional value of food due to the safety of these antioxidants and consumers prefer foods supplemented with natural antioxidants instead of synthetic ones (Velasco and Williams, 2011). The change from highly effective synthetic antioxidants to natural antioxidants is due to the fact that synthetic antioxidants can be toxic to humans and have carcinogenic side effects related to long-term consumption (Gharavi et al., 2007).

There are several natural antioxidants derived from fruits, vegetables, seeds, herbs, cereal, and spices that have been identified and approved for application in various food (Colindres and Brewer 2011; Naveena et al. 2013 Singh et al. 2014). The extraction of natural antioxidants from natural sources has been conducted by different means, including water, organic solvent, and aqueous solvents (Shah et al., 2014; Huang et al, 2018; Ghazya et al, 2021). Numerous plant extracts have been found to contain various anti-oxidative compounds such as phenolic acids, flavonoids, diterpenoids, and volatile compounds (Zheng et al., 2001; Shan et al., 2005). These antioxidative compounds isolated from these extractions are present in three forms, either as lipophilic, hydrophilic, and/or amphiphilic compounds (Baker et al., 2012). Total phenolic content, yield, and antioxidant capacity are strongly associated with organic solvents either independently or in combination with water (Baker et al., 2012; Shah et al., 2014). However, Shah et al. (2014), reported that ethanol is normally used for plant extraction, while the combination of organic solvent and water which may facilitate the recovery of compounds that are soluble in both solvent and water (Sutivisedsak et al., 2010; Puangsombat and Smith, 2010; Velasco and Williams, 2011). Hence, the application of a specific ratio of aqueous organic solvents depends mainly on the target antioxidant compounds and type of plant.

Anise (*Pimpinella anisum*) is widely used traditionally as a food additive and is considered a rich source of many compounds that pose high antioxidant activity (Sirini, et al., 2020; Zeng et al., 2014). According to Huang et al, (2018) most active compounds are anethole (90%), limonene (3.75%), and 4-allylanisole (1.5%). These compounds have been found to have the ability to scavenge free radicals, chelate metal, and reduce ferric antioxidant power (FRAP) that is responsible for oxidative rancidity (Shan et al., 2005; Velasco and Williams 2011; Liang et al., 2012).

Accordingly, to extract compounds that have hydrophilic and lipophilic properties the accurate ratio of ethanol solvent and water is needed (Wu et al., 2004). It has been recorded that different solvents have different activities compounds which are more related to their polarities and solute's chemical structure (Wu et al., 2004; Amer and Aly, 2019). Hence, most antioxidant compounds in anise are soluble in solvents and water like anethole compounds (Amer and Aly, 2019).

Moreover, the application of a certain organic solvent and plant organic solvent ratios that are appropriate for extracting all antioxidant compounds from all plants has not been demonstrated. Consequently, this study aimed to determine the effect of anise antioxidants extracted by different means on several quality parameters of broiler chicken meats when applied to meat post-slaughter.

## **MATERIALS AND METHODS**

### **Chemical materials**

Potassium hydroxide, chloroform, ethanol, malonaldehyde bis (diethyl acetal), trichloroacetic acid, and hydrochloric acid were obtained from Scharlab S. L, (Sentmenat, Spain) and phenolphthalein, 2-Thiobarbituric acid, from Chem-Lab NV, (Zedelgem, Belgium)

### **Preparation of Natural Antioxidant Extracts**

Dried anise seeds were obtained from commercial sources. Antioxidant extracts of anise seeds were produced using three separate solvent styles: 100% distilled water, 100% ethanol, and an aqueous ethanol mixture (ethanol:water at 70:30 v/v) according to the technique reported by Selani et al. (2011). One hundred grams of anise powder were mixed with either 1000 ml of water, ethanol, or aqueous ethanol. The mixtures were macerated for 48 h and stored at room temperature (20 °C) in a dark place. The extracts were then filtered using filter paper (Whatman® No. 1) and the filtrates concentrated at 60 °C in a water bath to get rid of excess solvent until a volumetric reduction of 80 % was obtained. The filtrates were then kept at -20 °C until subsequent use.

## Preparation of samples

This experiment was designed as a factorial design comprised of a  $4 \times 3$  with four treatments (water extract 100%, ethanol 100%, aqueous ethanol (ethanol 70% and water 30% and a non-treatment control), and three storage periods (0, 3, and 7 days). Chicken breast muscle samples were obtained from slaughterhouses and the chicken meat was minced by a mincer. Meat samples were divided into four treatments. Three experimental treatments were added 1% of water, ethanol, and aqueous ethanol (ethanol: water at 70:30 v/v) extract and mixed together, plus one treatment was not added extract (control). Meat samples were then wrapped with polyethylene bags and stored at 4 °C for 0, 3, and 7 days, afterward the meat samples were taken at each time of storage and measured for lipid oxidation, cooking loss, free fatty acids, and drip loss.

## Thiobarbituric acid reactive substances (TBARS)

TBARS value as a lipid oxidation indicator was determined in chicken meat samples according to the way initially reported by Buege and Aust (1978). Roughly 0.5 g of ground chicken meat was weighed and its weight exact weight recorded and placed in test tube (10 ml) and added 2.5 ml of TBARS stock solution (a stock solution that contained 3.75 g thiobarbituric acid, 150 g trichloroacetic acid, and HCl 0.25 M). All meat samples were vortexed for 15 seconds and placed in a water bath at 95 °C for 15 min, after which the test tubes were rapidly cooled down in cold tap water and centrifuged at 2500 g at 4°C for 10 min (K Centrifuge PLC Series, Taiwan). The supernatant was placed into a cuvette and the absorbance was measured by using a spectrophotometer (Jenway, 6300 spectrophotometers, UK) at 532 nm against a blank sample without a meat sample. The TBARS value in chicken meat samples was evaluated and expressed as mg of malondialdehyde equivalents/kg meat using an appropriate malondialdehyde standard curve.

## Drip Loss

The drip loss value of chicken meat was measured according to the procedure reported by Honikel (1998). Eighty grams of meat was weighed and located individually into a plastic net that hanged in the airtight container and then stored in refrigeration at 4 °C. The meat samples were removed from refrigeration after 24 h and dried by means of a paper towel and then reweighed. This result represented the '0 days. The same technique was applied on days 3 and 7. Later, the drip loss value was calculated by applying the following equation:

Drip loss value (%) =  $[(\text{Initial weight of raw meat (g)} - \text{final weight of meat (g)}) / \text{Initial weight of raw meat (g)}] \times 100$

## Cooking Loss

Approximately one hundred grams of raw meat was wrapped with aluminium foil and cooked at a temperature of 160 °C for 10 minutes in an oven. The meat was cooled to room temperature (23 °C) after the internal temperature of samples reached 71°C as determined by using a digital thermometer. The meat samples were dried with a paper towel and reweighed. The cooking loss of each sample was calculated by means of the following equation:

Cooking loss (%) = [(Initial weight of raw meat - weight of cooked meat)/ Initial weight of raw meat] ×100

## Free fatty acid

Free fatty acid (FFA) value in chicken meat samples was determined according to the technique reported by Rukunudin et al. (1998). Roughly 2.5 g of chicken meat sample was placed in a tube followed by adding 15 mL chloroform and mixed using a homogenizer for at least 1 min. The mixture of the sample was filtered by using filter paper (Whatman number-1). After that 10 mL of the filtrate was placed in a test tube followed by adding 5 drops of 1% ethanolic phenolphthalein used as an indicator then titrated with a 0.01 N of ethanolic potassium hydroxide solution. The free fatty acid value was measured using the following equation:

Free fatty acid (FFA %) = [((mL of titration × Normality of KOH × 28.2))/ (Initial weight of meat sample (g))] x100

## Sensory evaluation

Chicken meat samples either supplemented with anise extracts or without (control) were evaluated for sensory attributes including colour, juiciness, tenderness, flavour, and overall acceptability.

The sensory evolution of chicken meat either treated or without anise extracts was conducted in the meat technology lab at the animal production department. The evaluation was carried out on day five of the storage period by 7 trained panelists (2 females and 5 males) after recruitment, selection, and training. The meat muscle was cooked in an oven at a temperature of 176 °C for 9 minutes till the internal temperature reached 71 °C. Panelists then assessed the cooked meat's sensory attributes such as color, odor, flavor, tenderness, and juiciness. The samples were offered to panelists at 50 °C after being coded with three-digit random codes. The samples were presented to panelists every 4 minutes to give the panel time to assess the meat samples thoroughly. Distilled water was offered to panelists at room temperature and crackers to clean their palate from residual taste and flavor before evaluation and between samples. The scale used to evaluate each attribute of cooked chicken meat samples was 1-8 points scales

for each attribute which were 1 extremely, dark, intense, intense tender and juicy bland, bland, tough, dry, and 8 extremely intense, intense tender and juicy. Panelists were record their results on the form and once completed, the results will analysis.

### **Statistical Analysis**

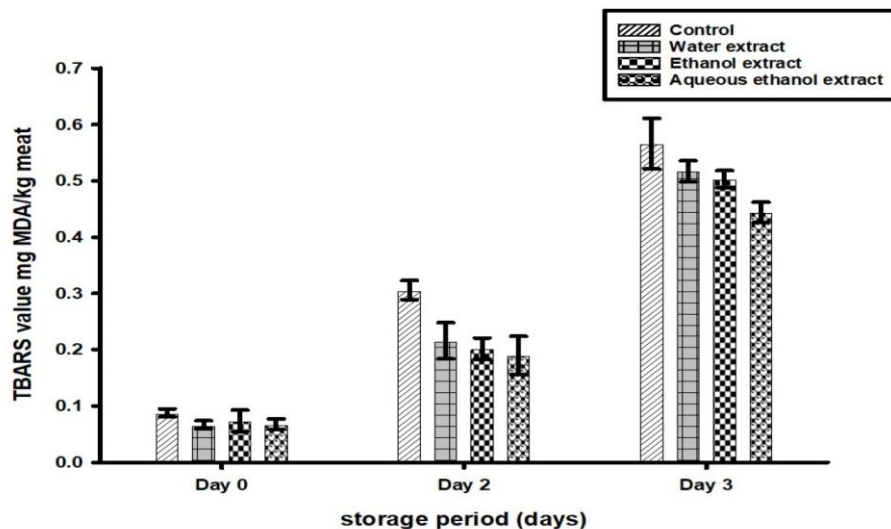
All data of this study were analyzed by using Genstat software (version 18, VSN International Ltd, UK). The data of lipid oxidation (TBARS value) and drip loss of chicken meat were analyzed by using a factorial design of a 4 x 3 where the four treatments (chicken meat treated with anise extracted by water, ethanol, an aqueous ethanol extracts, and control), and three refrigeration times (0, 3 and 7 days). The parameters (TBARS values, free fatty acids, and drip loss) were performed by using a two-way while the cooking loss was analyzed by using a one-way analysis of variance (ANOVA).

## **RESULTS AND DISCUSSION**

### **Effect of anise seed extracts by different means on lipid oxidation (TBARS value)**

The lipid oxidation (TBARS value) of chicken meat treated with anise extracts by different means was tested and shown in Figure (1). The storage time had a significant ( $P < 0.05$ ) effect on the TBARS value. Hence, the TBARS value was increased markedly in both treated and nontreated samples over 7 days. While inhibition of lipid oxidation was higher in treated samples at each point of storage time compared to the control (Figure 1). An elevation of TBARS values over the storage period in control samples is a good indicator of taking place lipid oxidation which is more likely related to the breakdown of hydroperoxides and producing malondialdehyde which is a secondary lipid oxidation product (Bax et al., 2012). It has been reported that an increase of TBARS in meat is indicative of advanced lipid oxidation that is responsible for off-odors and off-flavors which may have a negative impact on nutritional values, and sensory properties of meat, which lead to a decrease in the shelf-life of meat (Pegg and Shahidi 2007; Colindres and Brewer 2010). Similar results were detected by Kanattn et al (2014) who observed an elevation of lipid oxidation in minced chicken meat during chilled storage for 10 days. Furthermore, supplementation of chicken meat with anise extracts significantly reduced TBARS value during the storage period ( $p < 0.05$ ). Hence, the lipid oxidation value was found lower in all treated samples compared to the control for 7 days of storage period. These findings were similar to that reported by Kanattn et al (2014) who observed that minced chicken meat treated with anise extract had the lowest TBARS value compared to the control over 10 days of storage time. These results were in agreement with those reported by Xu et al, (2022) who supplemented meat star anise dietary fiber which inhibited significantly lipid oxidation during chilled storage. Moreover, the lowest TBARS value observed in chicken meat samples were

treated with aqueous ethanol extract compared to the control and the other treatments over the refrigerated period. This may be due to aqueous ethanol that has both hydrophilic and lipophilic phenolic compounds which may have more potent antioxidant activities. Accordingly, to extract hydrophilic and lipophilic compounds the accurate ratio of ethanol and water is needed (Wu et al., 2004).

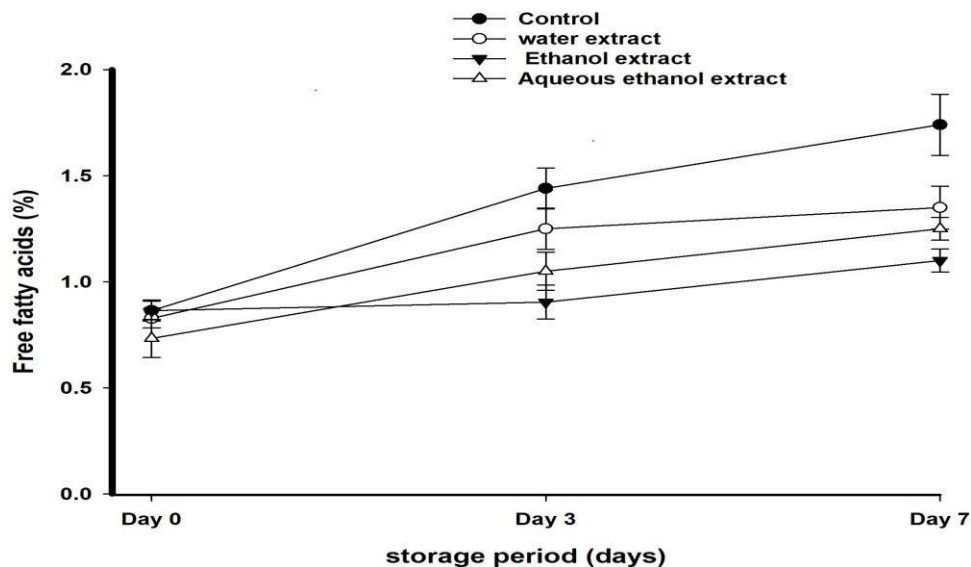


**Figure 1: Effect of Anise seed extracts on TBARS value (mg MDA/kg meat) of chicken meat. The data shown are the average and standard errors of differences in means**

It has been recorded that different solvents have different activities compounds which are more related to their polarities and solute's chemical structure (Amer and Aly, 2019). Hence, most antioxidant compounds in anise are soluble in solvents and water like anethole compounds (Amer and Aly, 2019). Huang et al (2018) found that the predominate component in anise is trans-anethole, which may be responsible for inhibiting the lipid oxidation process in meat samples used in the current study. Padmashree et al (2017) found that aqueous solvents had a third-fold more total phenols and flavonoids than water extract. They also found that ethanolic water extract inhibited lipid oxidation compared to the water and petroleum ether extract.

### Effect of anise seed extracts by different means on free fatty acids

The free fatty acids of chicken meat either treated with anise extracts or not are shown in (Figure 2). The storage time had a significant ( $P < 0.05$ ) effect on the formation of free fatty acids. Hence, the free fatty acids were increased markedly in both treated and non-treated samples over 7 days. While the highest formation of free fatty acids was observed in untreated samples at each point of storage time compared to treated samples (Figure 2). Free fatty acids are the products of lipid decomposition that are generated enzymatically and microbial which is a good indicator of the stability of lipids during the storage period (Al-doski et al 2020).



**Figure 2: Effect of Anise seed extracts on free fatty acids (%) of chicken meat. The data shown are the average and standard errors of differences in means**

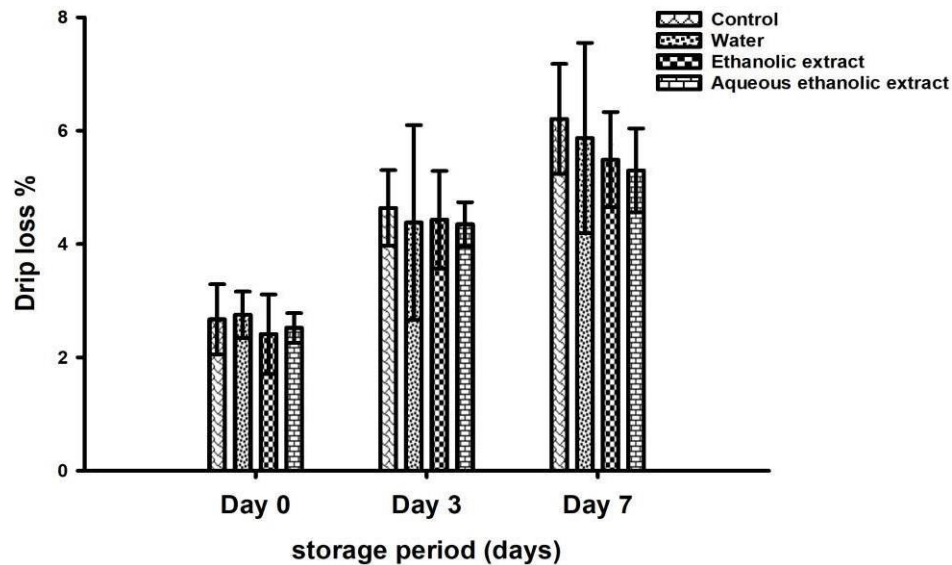
Chew and Nyam (2020) reported that free fatty acids act as pro-oxidants that speed up the decomposition rate of hydroperoxide which may lead to the development of an unpleasant taste and flavor in the meat. Similar findings were observed by Al-doski et al (2020) who recorded an elevation of free fatty acids in meat over storage time. Moreover, the results observed that the free fatty acids in meat samples were significantly affected by treatment ( $p < 0.05$ ). Hence, the amount of free fatty acid was lower in all treated samples at each point of storage time, while, the lowest value of free fatty acids was recorded in both meat samples that were treated with anise extracted by ethanol and aqueous ethanol compared to the control and water extracts respectively. This reduction of free fatty acids in meat may be due to the phenolic compounds that are present in anise which has more potent activity. Furthermore, no significant differences were found between ethanolic, water, and aqueous extracts in respect of free fatty acids content at days 3 and 7 (Figure 2).



### **Effect of anise seed extracts by different means on drip loss**

Meat samples that were supplemented with or without anise extracts showed an increase in **drip** losses over storage time (Figure 3). At 7 days of storage highest value of drip, losses were observed. An elevating of drip loss in chicken meat over the storage period is due to the oxidation of protein in meat, which could reduce the ability of meat proteins to hold water. Aaslyng et al. (2003) reported that drip loss can be defined as water losing in meat during the storage period or following a cooking process, which is mainly dependent upon the capability of myofibrillar protein to retain and bind water (Wang, et al., 2009). Hence, proteins in meat like fat, undergo an oxidation process by a free radical mechanism (Grossi, et al., 2014). Exhibition the largest percentage of drip loss is considered an undesirable impact on meat quality (Khurshid, 2016). According to the results reported by Wang et al. (2011), sarcoplasmic and myofibrillar protein solubility in meat decreased with an increase in time. Similar findings were reported by Maqsood, et al. (2015), who found progressively an increase of drip loss in camel meat under refrigeration temperature with increasing storage time.

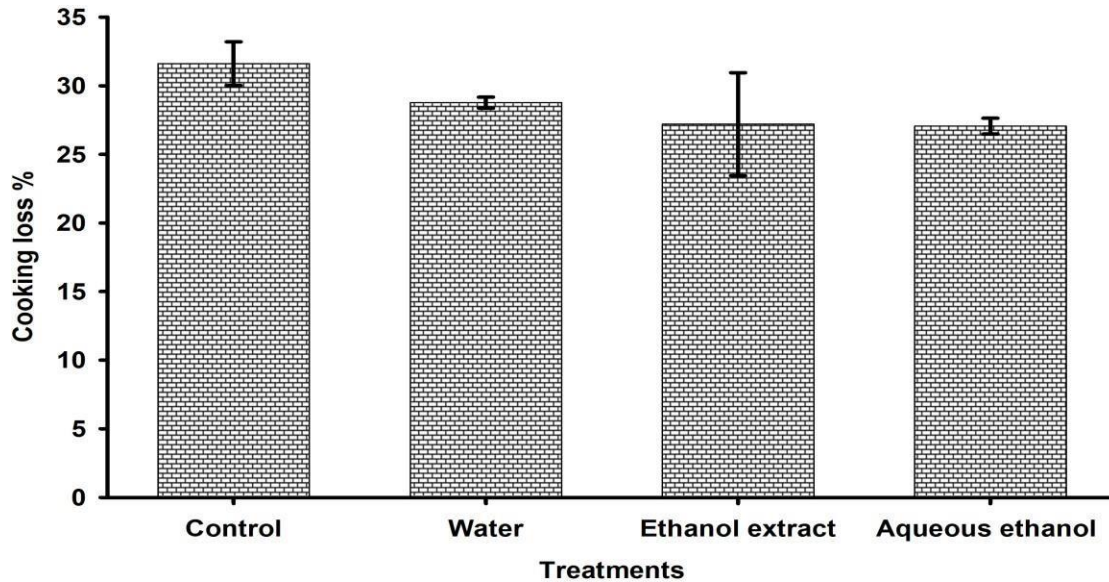
Meat supplemented with anise extracts reduced the drip loss level at each point of storage time compared to the control (Figure 3), however, differences in drip losses between the four treatments were not significant ( $P>0.05$ ). Moreover, aqueous ethanol extract was found to have the lowest drip loss over storage time compared to the other treatment at day 7 of storage time. It is possible that the inhibition of meat protein degradation by phenolic anti-oxidative compounds found in anise seed extracts enhances the capability of myofibrillar protein of meat to retain and bind more water (Wang, et al., 2009). Orlien et al (2014) reported that proteins, like triglycerides and phospholipids, undergo oxidation by a free radical mechanism. Hence, it is likely that all free radicals can be retarded by using antioxidants. As such our results might indicate that retaining the water holding capacity of meat could be due to the protective effect of anise seeds extract on protein stability.



**Figure 3: Effect of Anise seed extracts on drip loss (%) of chicken meat. The data shown are the average and standard errors of differences in means.**

### Effect of anise seed extracts by different means on cooking loss

Cooking loss is the loss of water that occurs in meat during the cooking process (Roldan et al., 2015), which is specifically linked to the thermal process (Aaslyng et al., 2003) which will denature and oxidize protein (Wang, Pan, & Peng, 2009). Thermal denaturation of meat will reduce the ability of meat proteins to retain water and maintain their structure (Aaslyng et al., 2003). In our work, the cooking loss of meat was significantly affected by treatments ( $p < 0.001$ ). Hence, the highest amount of cooking loss was observed in control meat at 31.61% compared to the water, ethanol, and aqueous ethanol of 28.77, 27.02, and 27.06 %, respectively (Figure 4). The lowest exhibition of cooking loss was detected in ethanol and aqueous ethanol treatment. This was mainly due to the inhibition of oxidative damage of protein by antioxidant compounds which increased the ability of meat to retain more water. These results were in agreement with those reported by Xu et al, (2022) who observed that supplemented meat with star anise dietary fiber significantly decreased cooking loss over refrigeration storage time.



**Figure 4: Effect of Anise seed extracts on cooking loss (%) of chicken meat. The data shown are the average and standard errors of differences in means**

### **Effect of anise seed extracts by different means on sensory evaluation**

The results of sensory evaluation of the chicken meat treated with anise extracts or without are presented in Table (1). The mean values of attributes such as color, tenderness, juiciness, flavor, and overall acceptability of the meat were evaluated. The control treatment without anise

Extract received the lowest color scores, however, both the control treatment and treatments with added anise extracts did not differ significantly ( $P>0.05$ ). Moreover, non-significant differences were detected between the four treatments for tenderness, juiciness, flavor, and overall acceptability as well ( $P>0.05$ ) (Table 1).

**Table 1 Effect of Anise seed extracts on sensory evaluation of chicken meat (Mean  $\pm$  SE) following five days of treatment.**

	Color	Tenderness	Juiciness	Flavor	Overall acceptability
<b>Control</b>	6.25 $\pm$ 0.25	7.25 $\pm$ 0.25	6.75 $\pm$ 0.25	7.25 $\pm$ 0.47	7.50 $\pm$ 0.28
<b>Water extract</b>	6.75 $\pm$ 0.25	7.50 $\pm$ 0.28	7.50 $\pm$ 0.28	7.25 $\pm$ 0.47	7.25 $\pm$ 0.25
<b>Ethanol extract</b>	6.75 $\pm$ 0.47	7.50 $\pm$ 0.28	7.00 $\pm$ 0.40	8.00 $\pm$ 0.25	7.25 $\pm$ 0.25
<b>Aqueous ethanol extract</b>	7.25 $\pm$ 0.47	7.25 $\pm$ 0.47	7.00 $\pm$ 0.40	6.75 $\pm$ 0.47	7.25 $\pm$ 0.47

#### 4. CONCLUSIONS:

The results of this work suggest that meat samples without anise extract added had low stability against oxidative damage during the storage period. Supplementation of meat with anise extract before storage time provided the greatest protection of lipids. Hence, the highest protection of lipids was detected in meat treated with anise extracted by aqueous ethanol as evidenced by the lowest TBARS value and free fatty acids observed. Meat treated with anise extracts had the highest capacity of holding more water as the lowest cooking loss and drip loss were detected compared to the control. These results recommend that anise extract as a natural antioxidant performed significantly as an inhibition product of lipid oxidation. Because of this, applying anise extracted by different means should be considered an efficient way to protect meat characteristics and its nutritional value of meat.

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