



Detection of active compounds in ginger and coriander extract using HPLC technique and studying their effect on improving the properties of frozen meat slices

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Abstract

The study included the possibility of using local plants that are inexpensive and rich in active compounds, namely ginger and coriander, to improve meat qualities. The yield percentage of ginger extract was higher than that of coriander extract, reaching 12.40%, while it was 7.10% in coriander extract. When separating and characterizing the extracts using HPLC technology, it was found that the ginger extract contained the active compounds Gallic Acid, Quercetin, Caffeic Acid, Pyrogallol, P-Coumaric, Hydrobenzoic acid, Ferulic Acid, while the coriander extract contained some compounds and lacked others. Among the compounds found in the extract are Caffeic Acid, P-Coumaric, Gallic Acid, Ferulic Acid. The results also showed when estimating the value of peroxide PV, thiobarbituric acid TBA, free fatty acids FFA, and total volatile nitrogen TVN, that each of them decreased by using plant extracts compared to the control sample, while it increased slightly with the continued storage period up to 30 days.

Keywords : ginger – coriander - HPLC technique - frozen meat slices

Introduction

Meat is one of the essential foods for humans. Because it is rich in proteins and vitamins, countries seek to provide these types of red and white meat to the consumer ((William, 2007, so the problem of preserving food products is one of the most complex problems due to the market's need for products that have a longer shelf life, as fatty oxidation is one of the main causes of food spoilage, which leads to changes in texture, taste and appearance, as well as nutritional benefit, which leads to reducing the shelf life of food, as hydroperoxides that represent the first product of the oxidation process are volatile and then decompose later, forming unstable secondary compounds with a strong and pungent taste such as aldehydes, ketones, polymers and organic acids (Sun, et al, 2011), so current studies have turned to the use of plant extracts, herbs and spices in the food manufacturing process due to their high content of phenolic compounds that act as antimicrobial and antioxidants, which increases the shelf life of food, in addition to the effectiveness of these compounds in tenderizing meat and increasing its palatability. Phenolic compounds are characterized by their effectiveness High antimicrobial and antioxidant properties due to the ability of phenolic compounds to interact with proteins and due to the presence of the hydrophobic Benzenoid ring and hydrogen bonds that give phenolic compounds the ability to inhibit enzymes that generate free radicals such as Lipoxigenase, Cyclooxygenase and Xanthine oxidase, which gives phenols the ability to act as antioxidants (Parr & Bolwell, 2002). These plant extracts can be easily obtained, they are inexpensive and can be used at home or industrial levels to improve the qualities of meat mentioned above, in addition to using them as antioxidants instead of synthetic antioxidants that cause cancer (Velasco & Williams, 2011). Many fruits, vegetables, spices and medicinal herbs contain many active biological compounds such as phenolic compounds, flavonoids and tannins that have the ability to capture free radicals and act as natural antioxidants, in addition to their effectiveness as antimicrobials, which increases the shelf life of meat. Plants are also important food sources for the human body; because they contain carbohydrates, vitamins, minerals, organic acids and phenolic compounds, which make them play an important role in reducing the risk of many diseases such as



cancer, heart disease, diabetes, nervous system diseases and other diseases (Al-Halfi 2009). This is what encouraged this study to benefit from coriander and ginger seeds and their extracts in extending the storage life and improving the sensory qualities of meat.

I. Materials and Methods

Preparation of extracts:

The extracts of ginger and coriander were prepared according to the method described by Pin-Der and Gow-Chin (1997). 10 g of the plant was weighed with 300 ml of distilled water at boiling point and left for 30 minutes on a magnetic mixer, filtered through filter paper (Whatman No. 1), then concentrated by rotary evaporator at a temperature of less than 70 °C. The concentrated extract was then poured into a Petri dish and placed in an electric oven at a temperature of 40 °C/24 °C for an hour to dry. The dried powder was scraped off and collected in dry bottles and stored in the refrigerator until use.

Estimation of the percentage of extraction yield:

The extraction yield was calculated according to the method described by Al-Khafaji et al. (2009) as follows:

$$\text{Extraction yield \%} = \{ \text{weight of dry extract (g)} / \text{original weight of plant powder (g)} \} \times 100$$

High performance liquid chromatography (HPLC) technique:

This method was used to identify some active substances in ginger and coriander extract, and the same conditions mentioned by Mradu, et. al, (2012) were followed using a C18-ODS separation column (25cm × 4.6 mm) and the mobile phase methanol: water: formic acid in the proportions (70: 25: 5) v/v and at a flow rate of 1 ml/min, at a temperature of 30 °C, while the wavelength used was 280 nm. The test was done by taking 1 ml of the extract and adding 1 ml of the mobile phase to it. After mixing well using the Vortex mixer, 5 microliters of it were injected into the device and the retention time of the model compound was compared with the standard compound appearance time.

Preparation of meat slices and immersion in the extracts:

The meat was cut into slices with a thickness of 1.5-2 cm and a weight of approximately 150 g, then treated with plant extracts of ginger and coriander at concentrations of 2, 4 and 6%, in addition to distilled water and trypsin enzyme as a comparison treatment using the immersion method for two hours. After the immersion period was over, they were placed in polyethylene bags and frozen for 30 days. During this period, the changes in some chemicals were monitored during storage periods of 0, 15 and 30 days.

Chemical tests of meat slices during storage stages:

1- Estimation of total volatile nitrogen (TVN)

Total volatile nitrogen was estimated in meat samples according to the method mentioned (Al-Taie and Al-Moussawi, 1992), where 100 of the sample were homogenized with 300 ml of trichloroacetic acid solution with a concentration of 5%, then the mixture was filtered to obtain a clear extract, then 5 ml of the clear extract was transferred to the distillation flask (Kjeldahl) and 5 ml of sodium hydroxide solution with a concentration of 2 molar was added to it, then the Kjeldahl apparatus was connected and the mixture was heated, the distilled liquid was received in the receiving flask, as it contains 15 ml of boric acid with a concentration of 4% added to it drops of methyl red dye indicator and bromocresol green, then the mixture was calibrated using sulfuric acid with a concentration of 0.01 molar and the amount of total volatile nitrogen was calculated according to For the following equation:

TVN (mgN/100gm) = Titration (ml (0.1N) H₂So₄) X 14

2- Estimation of Peroxide Value (PV)

The method mentioned in A.O.A.C (2010) was used to estimate the peroxide value in frozen meat samples, and this is done by mixing 5 g of the sample with (15) ml of distilled water for one minute, then (20) ml of Sodium dodeceyl Sulphate was added at a concentration of (0.1) molar with stirring for two minutes, then ((40 ml of ethanol was added, the solution was shaken well, then 20)) ml of hexane was added, then placed in a centrifuge (1000) rpm for (20) minutes, then the oil layer formed on the liquid surface was separated and ground with potassium hydroxide (0.01) molar, and the values were calculated in milliequivalent kg.

Number of milliliters of potassium hydroxide X N

Peroxide value = $\frac{\text{Number of milliliters of potassium hydroxide X N}}{\text{weight of the sample}} \times 100$

Milliequivalents/kg meat

3- Estimation of Thiobarbituric acid (TBA)

Fat oxidation in meat samples was measured by estimating thiobarbituric acid according to the method of Witte, et. al, (1989)), where 1 g of meat sample was homogenized with 25 ml of a cold solution containing 20% Trichloro acetic acid (TCA) dissolved in phosphoric acid with a concentration of 2 M, which was placed in the homogenizer for 2 minutes, then the mixture was transferred to a 50 ml volumetric flask, and the volume was completed to the mark with distilled water, the mixture was shaken and 25 ml was taken from it and centrifuged at a speed of 3000 rpm for 30 minutes, then the mixture was filtered with filter paper, after which 5 ml of the filtrate was transferred to a test tube and 5 ml of thiobarbituric acid reagent solution with a concentration of 0.005 M was added to it, and Blank's solution was prepared by mixing 5 ml of distilled water with 5 ml of the reagent solution, the contents of the test tubes were mixed well, tightly closed and stored in a dark place for 15-16 hours at room temperature, and the contents were heated in a water bath for 35 minutes, after which the absorbance (A) of the resulting color was measured at a wavelength of 530 nm using a spectrophotometer, then the value of thiobarbituric acid was calculated by multiplying the absorbance value by the factor 5.2 and was based on mg malondialdehyde / kg meat and according to the following equation

Thiobarbituric acid value (mg malon aldehyde / kg meat) = 5.2 X A

4- Estimation of free fatty acids Free Fatty Acid (FFA)

The acid value was calculated and from it the percentage of free fatty acids was calculated according to the method mentioned by Al-Taie and Al-Moussawi (1992), as 25 ml of ethyl ether were mixed with 25 ml of 98% ethyl alcohol with 1 ml of 1% phenolphthalein solution, then it was accurately balanced using a 0.1 standard basic solution, then 10 g of the sample was taken and placed in the prepared solution and filtered. Then the filtrate was graded with 0.1 N sodium hydroxide until the pink color appeared, which remained stable for 15 seconds. Then the acid number was calculated as follows:

Number of milliliters of sodium hydroxide X 5.61

Acid number = $\frac{\text{Number of milliliters of sodium hydroxide X 5.61}}{\text{Sample weight (mg)}}$

Sample weight (mg)

Acid number

$$\text{Free fatty acids \%} = \frac{\text{—————}}{2}$$

Results and Discussion:

Extraction Yield Percentage:

The results in Figure (1) show the percentage of the extraction yield of plant extracts using distilled water. It is noted from the figure that the ginger extract is superior in the percentage of the extraction yield, which reached 12.4%, while the coriander extract reached 7.1%. The ginger extraction percentage was less than what was reached by (spyrour, et.al, 2024) when extracting ginger with different types of solvents and using the saxolite device. The percentage of the yield for each extract was calculated, and water was superior to the other solvents in the percentage of the extraction yield, which reached 17.93%, followed by the ethanol solvent, then ethyl acetate, then hexane, which reached an extraction percentage of 17.70, 8.28, 4.82%, respectively. The reason for the difference in the extraction yield percentage may be due to several reasons, the most important of which is the difference in the method used for extraction, as well as the extraction period and the efficiency of the devices, in addition to the difference in the chemicals or solvents used in the extraction process and the distribution of materials and nutrients on the part exposed to extraction. Also, loss may occur The extract when skimming the extracts after the drying process is complete and the nature of the chemical compounds present in the plants and their ability to dissolve or not dissolve and their attraction by the solvent (Al-Haluj, 2009). The method of collecting, picking, cleaning, grinding, exposure to sunlight and preservation of samples, in addition to the difference in geographical location, climate, irrigation and irrigation water content (Najah, 2019), all of this affects the type and quantity of the product and then affects the type and quantity of active compounds in the extract, as it means many factors that affect the quantity and quality of the extracted phenolic compounds, including the method used in extraction, the solvents used, the size of the particles of the materials, the time and temperature of extraction and the degree of polarity of the extracted phenolic compounds, as well as the degree of oxidation of the compounds to be extracted.

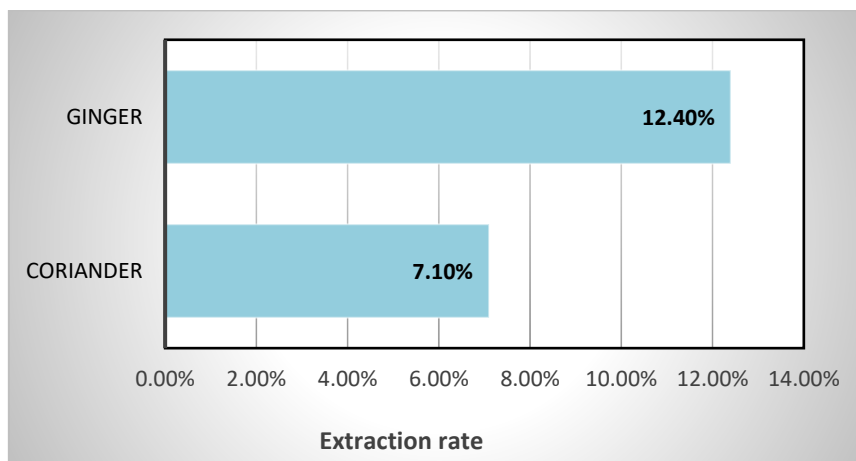


Figure (1): The percentage of water extraction of ginger and coriander plants

High Performance Liquid Chromatograph (HPLC)

The results shown in Table (1) and Figures (2) and (3) showed the separation and identification of aqueous extracts of ginger and coriander using HPLC technology in the presence of a group of standard



compounds Figures (4), namely Gallic Acid, Quercetin, Caffeic Acid, Pyrogallol, P-Coumaric, Hydrobenzoic acid, and Ferulic Acid. Figure (3) shows the appearance of seven peaks for the ginger extract when separated by HPLC technology, the retention time of which matches the retention time of the peaks of the standard compounds. The data shown in Table (1) show that the ginger extract contains the following compounds: Pyrogallol, Hydrobenzoic acid, Quercetin, and a concentration of 22.65, 16.28, and 19.80 ppm, respectively, while the coriander extract lacks these compounds. It is also noted from the same table that the ginger extract is superior to the coriander extract in containing the Ferulic compound. Acid and Gallic Acid, which reached 30.25, 35.65 ppm in ginger extract, respectively, while it was recorded in coriander extract 25.98, 30.65 ppm, respectively, and coriander extract excelled in containing P-Coumaric acid and Caffeic Acid, which reached 18.77, 22.25 ppm, respectively, while it was in ginger extract 12.78, 21.22 ppm, respectively, so we conclude from the results of this study that ginger contains most of the phenolic compounds, and this was confirmed by (Al-Areer, et.al, 2023) when separating the aqueous extract of ginger using HPLC technology, as he found a group of separated compounds, including Gallic Acid, Caffeic Acid, Quercetin, and Rutin, with a concentration of 1.61, 1.05, 1.49, 11.96 ml/mg, respectively.

Table (1): Concentration of active compounds separated by HPLC technique from ginger and coriander extract

No	Compounds (PPM)	Ginger	Coriander
1	Pyrogallol	22.65	-----
2	Hydrobenzoic acid	16.28	-----
3	Ferulic acid	30.25	25.98
4	p-coumaric acid	12.78	18.77
5	Gallic acid	35.65	30.65
6	Caffeic acid	21.22	22.25
7	Quercetin	19.80	-----

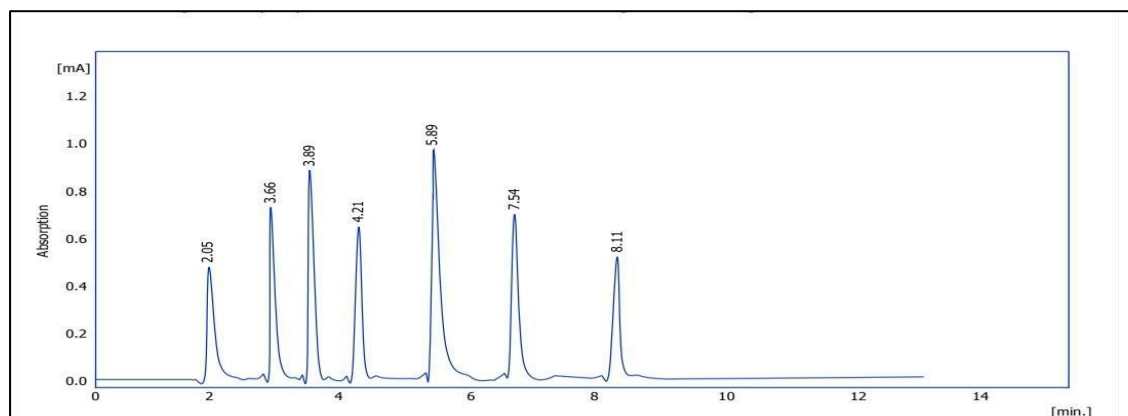


Figure (2): Chromatogram showing the concentration of active compounds using HPLC technology for coriander extract.

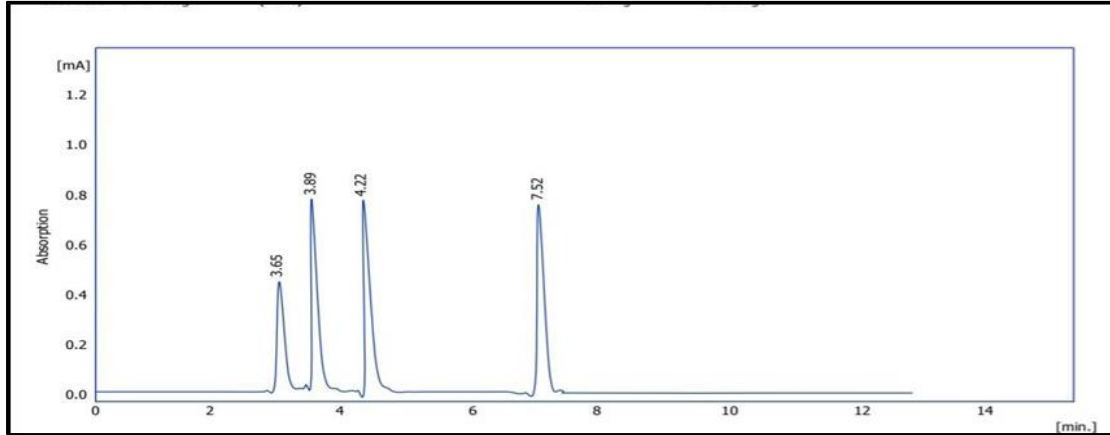


Figure (3): Chromatogram showing the concentration of active compounds using HPLC technology for ginger extract.

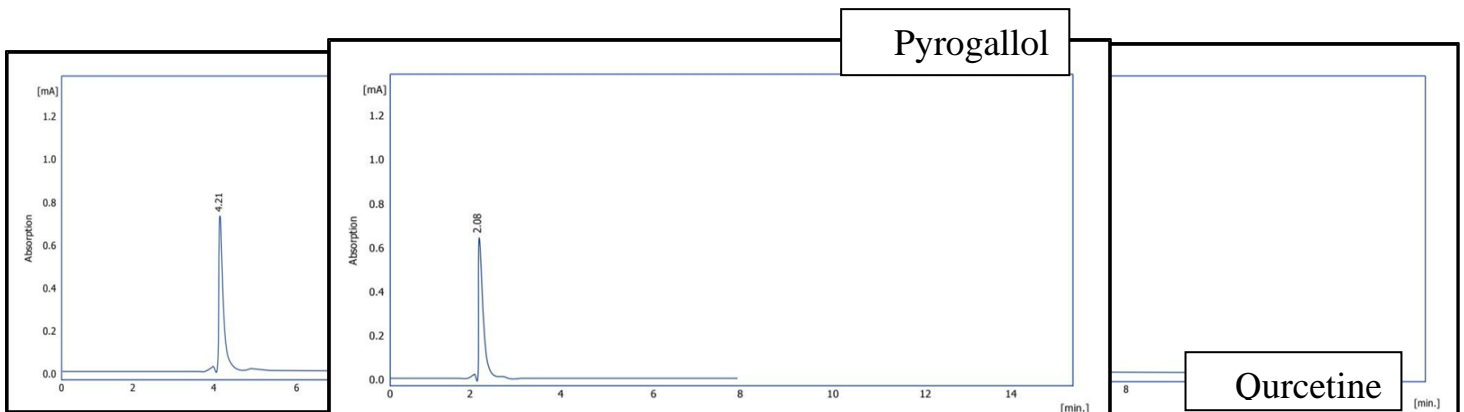
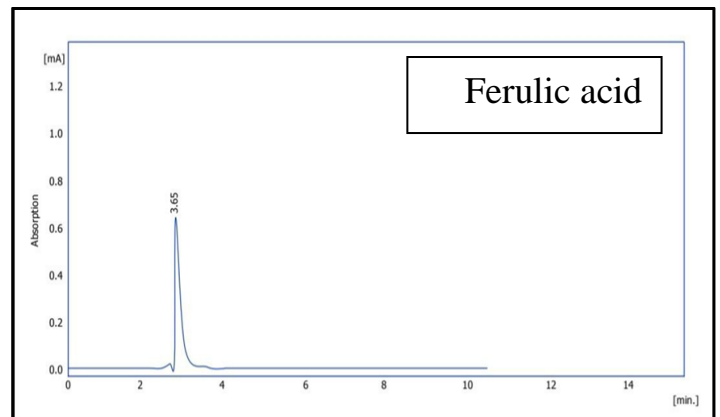
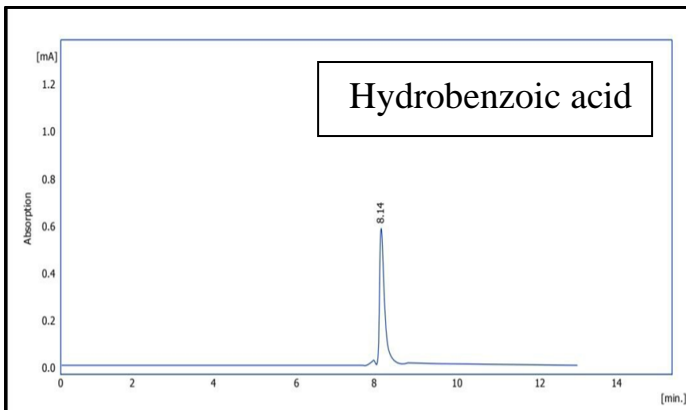


Figure (4): Chromatogram showing the concentration of the standard compounds using HPLC technique.

Effect of plant extracts on total volatile nitrogen TVN mg/100 N/g meat in frozen stored beef slices.

Figure (5) shows the values of total volatile nitrogen (TVN) for meat slices treated with plant extracts compared to ascorbic acid before and during storage. The results showed that the highest percentage of total volatile nitrogen was in the control sample using distilled water for all storage periods 0-30.15 days, reaching 6.94%, 5.13%, 4.50 mg/N 100 g meat, respectively, compared to the treatments in which the extracts under study were used. The TVN value decreased significantly, as the treatment of meat slices with ginger extract 6% recorded the highest decrease compared to the other extracts and for all storage periods, recording 4.20%, 3.94%, 3.68 mg/N 100 g meat for the period 30.15 days, respectively, while the coriander extract treatment for the same concentrations recorded 6.4.2%, 3.83%, 4.04, 4.23 mg 100/Ng meat respectively at the beginning of storage and increased slightly at the end of storage until it reached 4.81%, 5.72, 6.75 mg/N100 respectively after 30 days, while the ginger extract treatment at concentrations of 6, 4, 2% recorded TVN values of 3.68%, 3.82 and 4.04 mg/N100/g meat respectively at the beginning of the storage period, while it recorded 4.20%, 4.82 and 5.36 mg/N100/g meat respectively at the end of the storage period after 30 days of storage, while the ascorbic acid treatment recorded TVN values of 4.70%, 3.76 mg/N100/g meat at the beginning of storage 0 days and after 30 days respectively.

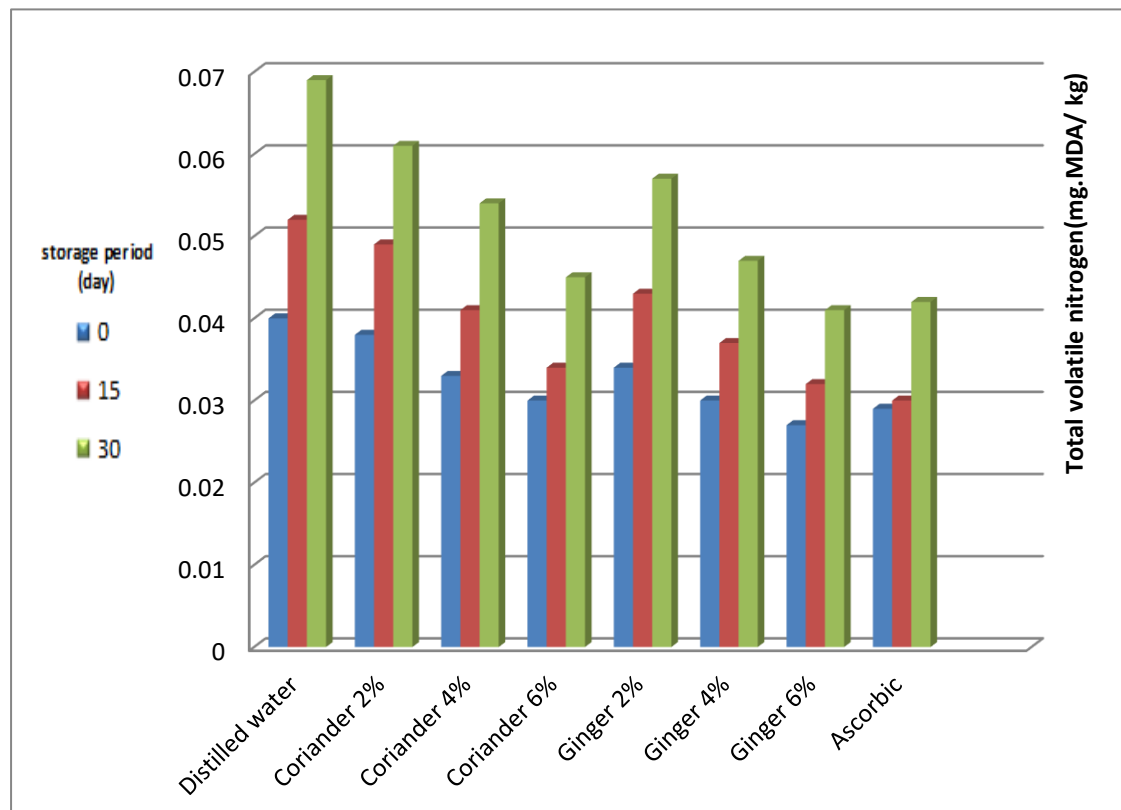


Figure (5): The effect of treatment with plant extracts on the amount of total volatile nitrogen mg N/100 g of meat in frozen beef slices

The results of this study conclude that total volatile nitrogen TVN decreases with increasing concentration of tested plant extracts, in addition to that the TVN value decreases in meat slices treated with plant extracts compared to the high TVN percentage of the control sample using distilled water. This is due to the plant extracts containing active compounds that have an effective effect on antioxidants that work to oxidize meat during freezing storage. As for the effect of the storage period on the TVN percentage, meat slices treated with plant extracts have an insignificant effect for treatment with plant extracts, as it increased for all treatments in this study, but it was a slight increase, as the increase in the TVN percentage was a slight increase, and this may be due to the presence of active compounds that work as antioxidants. This was confirmed by Al-Zubaidi et al. (2009) that coriander extract contains tannins, phenols, glycosides and flavones that have an effective effect on oxidants. The reason for the increase in the TVN value with the continued storage period is the result of the increased activity of some microorganisms that work to produce protein-degrading enzymes, and then as a result of protein decomposition, some compounds are released. Which leads to an increase in the percentage of TVN (Zhang, et al., 2018; Miao, et al., 2015; Daniela, et al., 2013). The most important of these compounds are (TMA) Trimethyl amine and ammonia NH₃, which are formed by the growth of some types of bacteria that cause spoilage of refrigerated meat. The compound (DMA) Dimethylamine is also produced by autolytic enzymes during refrigerated storage. These compounds cause an increase in the percentage of total volatile nitrogen TVN when the storage period increases (Miao, et al., 2015). The results agreed with what Al-Taie (2021) and Mahoud (2022) reached, as they found that the storage period has an effect on the values of TVN. It was at its lowest level at the beginning of the storage period of 0 days, then it begins to rise with the progress of storage until it reaches its highest level at the end of the storage period. These results also agreed with the Iraqi standard specification issued by the Central Organization for Standardization and Quality Control No. 2688 for the year 1987, which stipulates that the TVN percentage should not exceed 14% mg/100 N/g of meat, and therefore it is considered unacceptable.

Effect of plant extracts on peroxide values (mEq/kg) in frozen beef slices.

Figure (6) shows the peroxide values of the meat slices treated with plant extracts before and during storage. The results showed a decrease in the peroxide values in the meat slices treated with plant extracts, but this decrease varied according to the type of extract. The lowest percentage of peroxide was in the meat slices treated with ginger extract 6%, while the highest percentage of peroxide was in the control treatment (distilled water treatment), as the percentage of peroxide in the coriander treatment reached 6% and distilled water at the end of the storage period after 30 days 5.11%, 4.00 mEq/kg oil, respectively. It was also noted that the peroxide values decrease with increasing concentration of the extract, which was confirmed by Al-Qatini (2019) that during the storage period, fat oxidation occurs and then peroxides are formed, which increase during the increase in the storage period, but treatment with extracts reduces the formation of peroxides due to their containing active compounds that inhibit the formation of these peroxides. This is consistent with what Al-Zubaidi et al. concluded. (2009)) When studying the effect of aqueous and alcoholic extracts of coriander and dracaena seeds as antioxidants in minced meat fat and stock, as the alcoholic extract of coriander seeds has great effectiveness in preventing the oxidation of minced meat fat due to the active compounds it contains such as tannins, glycosides, phenols and flavones.

It was also noted that there was an increase in peroxide values with the progress of the storage period and for all treatments, but it was less in the plant extract treatments, as the peroxide values increased in the coriander extract treatments for concentrations of 2, 4, 6%, reaching 4.17, 3.94, 3.54% milliequivalent/kg oil, respectively, to 4.25%, 4.92%, 5.07 milliequivalent/kg oil, respectively, at the end of the storage period after 30 days, while the values in the ginger extract treatments for concentrations of 6%, 4%, 2% at the beginning of storage reached 3.17%, 3.57%, 3.84 milliequivalent/kg oil, respectively, then reached 4.00%, 4.41%, 4.80 milliequivalent/kg oil, respectively, and the peroxide values were recorded in the treatment with distilled water and ascorbic acid at the beginning of Storage %3.40, 4.32 mEq/kg oil respectively, while at the end of the storage period it reached %4.29, 5.11 mEq/kg oil respectively, and these results are consistent with the Iraqi standard specification issued by



the Central Agency for Standardization and Quality Control No. 2688 of 1987 for frozen and chilled red meat and poultry products, as the permissible peroxide value should not exceed 15 mEq/kg oil, and the results of this study agreed with what was reached by Mahoud (2022) and Al-Mashaikhi (2021) that the increase in peroxide values in minced meat discs with the advancement of the freezing storage period in the control sample is more than it is in meat discs treated with plant extracts.

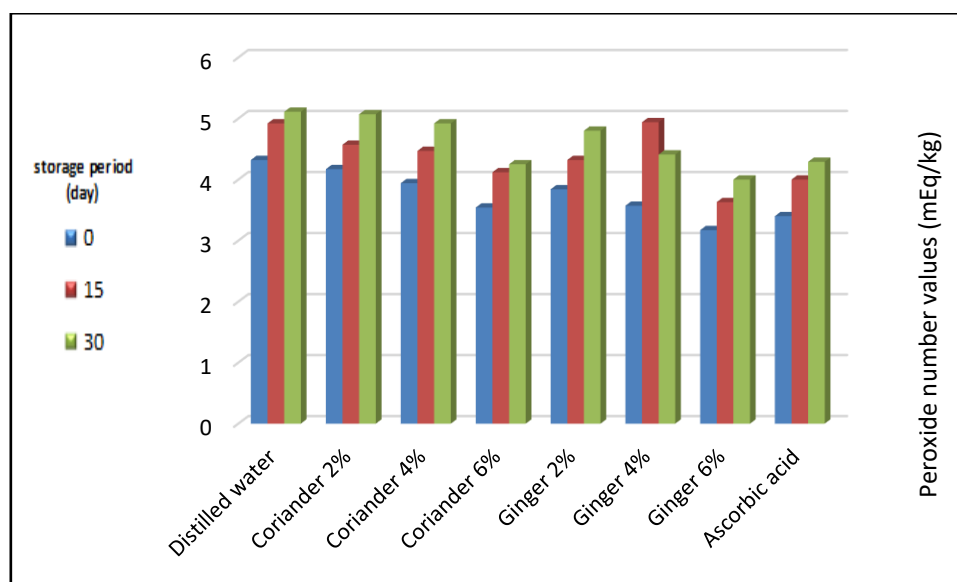


Figure (6) Effect of treatment with plant extracts on peroxide number (mEq/kg meat) in frozen beef slices.

Effect of plant extracts on thiobarbutyric acid and malondialdehyde (TBA) values/kg meat in frozen stored beef slices.

Effect of plant extracts on thiobarbutyric acid and malondialdehyde values/kg meat in frozen beef slices. Figure (7) shows significant differences between the TBA value of the meat slices treated with plant extracts compared to the control treatment. This difference in TBA values depends on the type and concentration of the extract added to the meat slices. The control treatment recorded the highest TBA value at the beginning of storage, which amounted to 0.040 mg malondialdehyde/kg of meat, while the ginger extract treatment with a concentration of 6% outperformed the TBA value, which increased slightly and insignificantly during the storage period, reaching 0.027 mg malondialdehyde/kg of meat at the beginning of storage to reach 0.041 mg malondialdehyde/kg of meat at the end of storage. In general, the TBA values for all meat treatments with plant extracts increased insignificantly compared to the control treatment. This may be due to the fact that these extracts contain active compounds such as phenols, which protect fats from oxidation and thus reduce rancidity or delay the occurrence of rancidity, because these compounds interact with peroxide radicals through It was given a hydrogen atom from the phenolic hydroxyl group, and thus it is similar to tocopherols (Mahood 2022). As for the effect of the storage period in the extract treatments, there was an effect of the storage period on the TBA value, which continued to rise throughout the storage period, but this increase was slight and insignificant compared to the control treatment, in which the increase was significant. However, the treatment of ginger and coriander extract at a concentration of 2% had a clear increase in the TBA percentage during the storage period, which showed that the concentration ratio of the extracts had a clear and effective effect, as the TBA percentage at the beginning of the storage period of 0 days for the treatment of coriander and ginger extract at a concentration of 2% reached 0.034%, 0.038 mg malondialdehyde/kg of meat, respectively, to rise significantly at the end of the storage period after 30 days to reach 0.057%, 0.061 mg malondialdehyde/kg of meat, respectively, while the TBA percentage in the coriander extract treatments

for concentrations of 6.4% was recorded in At the end of the storage period, the values of TBA were 0.045%, 0.054 mg MDA/kg meat, respectively, while the values of ginger extract for the same concentrations were 0.041%, 0.047 mg MDA/kg meat, respectively, and 0.042% mg MDA/kg meat for ascorbic acid treatment at the end of the storage period. The reason for the increase in TBA values with increasing storage period may be due to the oxidation of fats during it, which leads to the production of peroxides, aldehydes and ketones.

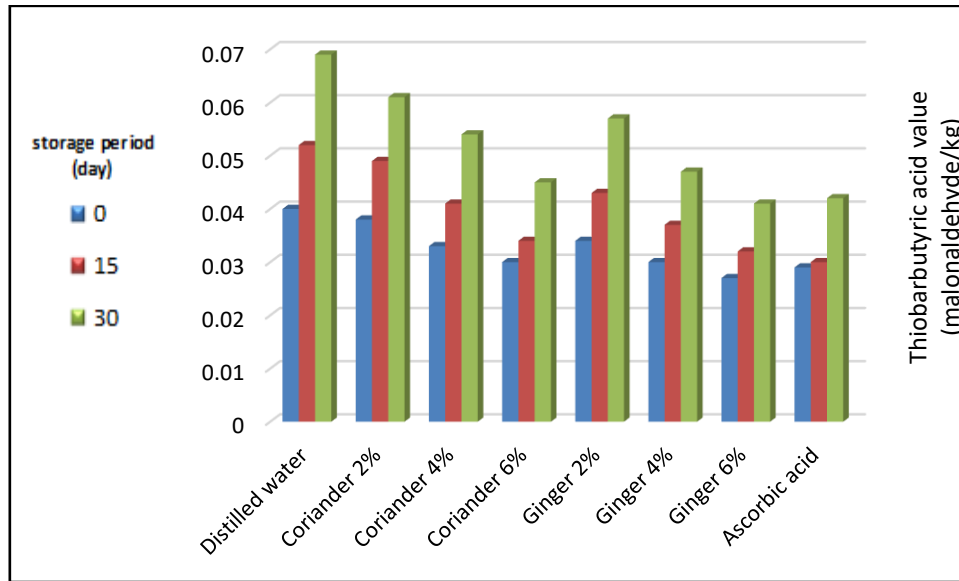


Figure (7): Effect of treatment with plant extracts on the value of thiobarbutyric acid (malonaldehyde/kg) in frozen beef slices.

The results of this study agreed with what Youssef (2014) observed in the increase in TBA values during the storage of frozen meat discs with added plant extracts, as the increase in TBA in these treatments was less compared to what it was for the control treatment. These results also agreed with the Iraqi standard specification issued by the Central Agency for Standardization and Quality Control No. 2688 of 1987, which stipulated that the TBA value for chilled and frozen red meat and poultry products does not exceed 2.0% mg malondialdehyde/kg meat and is considered rejected if it exceeds this percentage.

Effect of plant extracts on the percentage of free fatty acids (FFA) in frozen beef slices.

The effect of plant extracts on the percentage of free fatty acids (FFA) in frozen beef slices. Figure (8) shows the percentages of free fatty acids (FFA) for beef slices after treatment with plant extracts. The results showed a difference in the FFA percentages for all tested treatments at the beginning of the storage period of 0 days. This difference was different according to the type and concentration of the extract used in this study. The FFA percentage increased significantly in the control sample using distilled water compared to the FFA percentage for beef slices treated with extracts, in which the FFA percentage was lower than the distilled water treatment. It reached 0.69% at the beginning of storage for the control treatment, while the coriander treatment recorded 0.59, 0.52, 0.47% FFA percentage at the beginning of storage and for all tested concentrations 2, 4, 6% respectively. The percentages in the ginger treatment were 0.50, 0.43, 0.40% respectively for the concentrations mentioned above. As for the ascorbic acid treatment, its FFA percentage reached 0.43% at the beginning of the storage period. It is noted from Figure (8) that the percentage of fatty acids FFA increased with the advancement of the storage period in frozen meat slices for all treatments. Its values increased in the control treatment at the end of the storage period after 30 days to reach 0.89%. As for the meat slices treated with extracts, the

increase in fatty acids was less than the control treatment, as the percentage of FFA for the coriander treatment reached 2, 4%, in which the increase in FFA was significant but less than the control treatment 0.83, 0.75% respectively after 30 days of storage, while the increase in the percentage of FFA at the end of the storage period was not significant for the coriander treatments 6% and ginger for concentrations 2, 4, 6%, as it reached 0.60, 0.69, 0.62, 0.57% respectively, and the percentage of FFA for the ascorbic acid treatment reached 0.58% at the end of the storage period.

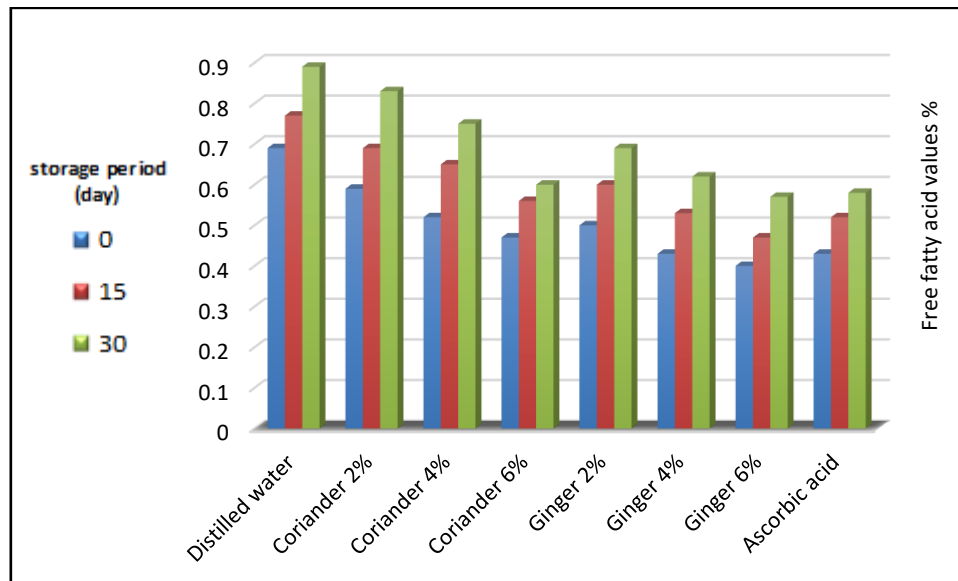


Figure (8) Effect of treatment with plant extracts on the percentage of free fatty acids in frozen beef slices.

We conclude from the above results that the percentage of fatty acids in the meat slices treated with plant extracts increased, but the increase was less compared to the control treatment, which was at its highest during the storage period. The reason for the difference in the percentage of free fatty acids between the meat slices to which plant extracts were added is the difference in the type of added extracts and what they contain of phenolic and flavonoid compounds and their effective effect as anti-microorganisms that decompose fats (Khan, et.al, 2009). Also, the reason for the increase in free fatty acids with the advancement of storage is due to the presence of lipolytic enzymes produced by microorganisms such as lipase and phospholipase, which work to release FFA, which leads to the production of an unacceptable odor that is reflected in the deterioration of the flavor with the increase in the storage period (Al-Rawi, 2005) (Al-Rubeii, et.al, 2009).

The results of this study agreed with the results of Al-Qatifi's study (2019) when adding plant extracts to the burger mixture, as he noticed an increase in the percentage of fatty acids for the burger treated with extracts compared to the control treatment, but this increase was slight compared to the control treatment in which the percentage of fatty acids was at its highest throughout the storage period. The results of this study also agreed with the Iraqi standard specification issued by the Central Organization for Standardization and Quality Control No. 2688 of 1987, which indicated that the percentage of free fatty acids should not exceed 1.5%, and if it is more than that, it is considered rejected.

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