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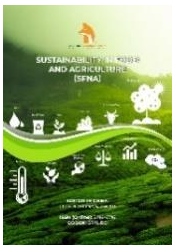

RESEARCH ARTICLE

A STUDY OF THE EFFECTIVENESS OF QUERCETIN COMPOUND ON THE QUALITATIVE PROPERTIES OF REFRIGERATED MEAT SLICES

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ABSTRACT

The current study was conducted at the Scientific Research Authority Laboratories / Environment and Water Research Center, aiming to evaluate the effectiveness of the natural quercetin compound extracted from chia seeds (*Salvia hispanica L.*) on the qualitative, sensory, and microbiological properties of refrigerated chicken breast fillets. This research responds to the modern trends of using safe, natural alternatives to chemical preservatives by employing bioactive phenolic compounds as antioxidants and antimicrobial agents. The quercetin compound was extracted using multi-stage extraction techniques, including the use of organic solvents (chloroform, butanol, and ethyl acetate), ultrasound, rotary evaporation, followed by concentration and purification stages. Solutions of the extract were prepared at different concentrations (1%, 2%, 3%), and chicken breast fillets were soaked in these solutions. Samples were then stored in vacuum bags at 4°C for periods of 0, 7, and 14 days. Several laboratory analyses were performed, including chemical analysis using HPLC to identify phenolic compounds, DPPH assay to measure antioxidant activity, microbiological tests including total aerobic bacterial count, *Salmonella* count, yeasts and molds counts, as well as sensory evaluation using a Likert scale assessing color, flavor, juiciness, tenderness, and overall acceptability. The results showed that adding quercetin to chicken breast fillets clearly improved sensory attributes, especially in sample (A3) at 3% concentration, which maintained acceptable tenderness, flavor, and color compared to the control (C). The antioxidant activity increased with quercetin concentration, with the 3% concentration achieving more than 87% free radical inhibition (DPPH). Quercetin significantly reduced microbial growth including aerobic bacteria, yeasts, molds, and *Salmonella*, with statistically significant differences especially on day 14 of storage. The best results were observed in samples (A3) and (A2) at 3% and 2% concentrations respectively, indicating a positive correlation between quercetin concentration and quality improvement indicators. These results undoubtedly uncover the importance of quercetin taken carefully from chia seeds as a promising natural ingredient that can be used to preserve fresh meat, effectively improve its quality without chemical additives, functioning as both an antioxidant and antimicrobial agent. It strikingly contributes to extending shelf life and highly improving qualitative properties without negatively affecting safety or sensory acceptance. Nonetheless, it preliminarily represents an economical and safe option in the food industry, going hand in hand with global trends toward clean and natural foods.

KEYWORDS

Quercetin, Chia Seeds, Natural Antioxidants, DPPH, and HPLC

1. INTRODUCTION

Principally, preserving fresh meat undoubtedly poses a significant noticeable challenge in the food industry, because meat is impressively highly susceptible to spoilage owing to chemical and microbial changes that take place when storing. Lipid oxidation and microbial growth principally lead to the exacerbation of meat quality properties, like tenderness, color, and flavor, as well as a reduction in shelf life (Zhou et al., 2010). This led the necessity of adopting natural compounds, like flavonoids, as effective antioxidants and antimicrobial agents. Quercetin, a natural compound belonging to the flavonoid group, is renowned for its maximal antioxidant and antibacterial properties, making it a sustainable option for improving the quality of food products (Boots et al., 2008). Chia seeds (*Salvia hispanica L.*) are considered a rich source of quercetin and other phenolic compounds, opening new avenues for the use of these natural sources in preserving fresh meat (Oliveira-Alves et al., 2020).

The importance related to quercetin embeds in its potentiality of enhancing food safety. Quercetin seemingly provides a natural alternative to synthetic additives, reducing contamination risks and promoting food safety. It also contributes to reducing meat waste, as quercetin helps in extending the shelf life of refrigerated meat slices, thus diminishing losing food and conclusively supporting the shift towards natural products. This research impressively supports using natural plant extracts as effective ingredients in the food industry. A research demonstrated quercetin's strong ability to reduce lipid oxidation, thereby slowing down the deterioration of food products by (Boots et al., 2008). According to the study, regarding the effects of natural compounds on meat quality, the use of natural extracts like quercetin highly boosts up the sensory attributes of meat and efficaciously reduces the formation of detrimental compounds when storing (Tang et al., 2021).

In the meantime, a research about chia seeds as a source of phenolic compounds carried out unveiled that chia seeds have high levels of phenols and quercetin, making them an ideal source of natural and effective antioxidant compounds (Oliveira-Alves et al., 2020). Numerous

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Iraqi studies have undoubtedly successfully extracted and identified quercetin out of local plant sources. In light of increasing health awareness among consumers, there is seemingly an imperative need to use natural and safe alternatives to traditional preservatives, a requirement requiring nutritional awareness to be sound and based on scientific principles, particularly in highly perishable animal products like meat (Jumaa and Nafi', 2014). Numerous modern research showed that plant-derived antioxidants, particularly flavonoids like quercetin, noticeably offer benefits beyond inhibiting oxidation. They also seamlessly suppress the growth of pathogenic microorganisms, contributing to extended shelf life and improved meat quality. To effectively avoid concerns over the negative effects of synthetic preservatives, research regarding using natural compounds as antioxidants in meat preservation has grown. Of these compounds, quercetin relatedly appeared as an effective agent derived from safe plant sources. Local studies, , have strikingly demonstrated the possibility of extracting quercetin from Iraqi plant peels such as (Mohammad et al., 2024). Furthermore, other Iraqi studies have efficaciously utilized bio-coating techniques with natural extracts or nanoparticles as alternative methods to maximally extend the shelf life of meat and effectively reduce spoilage when refrigerated (Saeed et al., 2023; Al-Ghanimi and Al-Rubeii, 2024).

These trends undoubtedly put a noticeable emphasis upon how important of expanding research into the effects of quercetin as a natural preservative is (Negi, 2012; Zhang et al., 2021). Quercetin is also renowned for its multiple effects on the sensory stability of food products, as it expressively helps maximally in maintaining the natural color and fresh flavor of animal products, particularly under cold storage conditions, an aspect that immensely enhances consumer acceptance (Gómez-Estaca et al., 2014).

Besides, the emergent interest in plant-based sources rich in phenolics, like chia seeds, intensely reflects a rising trend in both research and industrial applications owing to their numerous aspects of bioactive compounds with effective antioxidant and antimicrobial properties (Poudel et al., 2008). The addition of active plant compounds plays a role in extending shelf life while preserving the sensory quality of the product (Jumaa and Ghazal, 2010). Thus, using natural quercetin taken meticulously out of chia seeds highly represents a promising option within the international movement toward clean-label foods. Consumers increasingly and interestingly favor products free from synthetic chemicals and based on natural, scientifically validated ingredients (Asioli et al., 2017). Integrating such plant-based extracts into meat preservation technologies not only enhances food safety but also supports the food economy by reducing waste and improving the storage capacity of products.

2. Materials and Methods

2.1 Materials Used

The prime materials impressively adopted in the current experiment included chia seeds (*Salvia hispanica*), chloroform solvent, butanol, and ethyl acetate. The following equipment was also utilized: a mechanical shaker, ultrasonic bath, separating funnel, and rotary evaporator.

2.2 Extraction of Natural Quercetin from Chia Seeds

The quercetin extract out of chia seeds was meticulously prepared by the following steps:

- **Primary Extraction:** 30 grams of (*Salvia hispanica*) were finely ground, then meticulously mixed with 25 mL of chloroform. The mixture was placed on a mechanical shaker for 8hr at room temperature. After completion, the layer containing terpenes and fats was discarded.
- **Secondary Extraction:** The remaining seeds were treated with 100 mL of butanol and placed in an ultrasonic bath at 45°C for 15 minutes.
- **Separation and Purification:** The mixture was transferred to a separating funnel, and ethyl acetate was added. The funnel was shaken for 20 seconds. The resulting organic layer was separated, and the extraction process was repeated three times to ensure complete recovery of active compounds.
- **Drying:** The organic layer was dried using a rotary evaporator, yielding a concentrated quercetin extract.

2.3 Preparation of Chicken Breast Meat Slices

Fresh chicken breast meat (recently slaughtered and not frozen) was purchased from local markets in Baghdad. The meat was sliced into portions of approximately uniform weight, then soaked in quercetin extract solutions prepared at different concentrations (1%, 2%, and 3%) for 5 hours at refrigeration temperature (4°C). After soaking, the slices were gently dried using sterile filter paper, then packed in vacuum-sealed polyethylene bags using a vacuum packaging machine.

2.4 Storage

The treated and control samples were stored at 4°C for different time intervals (0, 7,14 days) for subsequent analysis.

2.5 Laboratory Analyses

A series of chemical, microbiological, and sensory analyses were conducted as follows:

- **Chemical Analyses:** These included the identification of phenolic compounds using High-Performance Liquid Chromatography (HPLC), in addition to the evaluation of antioxidant activity of the quercetin extract using the DPPH assay.
- **Microbiological Analyses:** These included total aerobic bacterial count, psychrotrophic bacteria, *Salmonella* bacteria, and yeast and mold counts using appropriate culture media and selective agar.
- **Sensory Evaluation:** A trained panel performed sensory evaluation of the samples in terms of color, flavor, and tenderness using the Likert scale of preference.

2.6 Extraction Method

A 30-gram sample was effectively and meticulously mixed with 25 mL of chloroform and imposingly placed on a shaker for 8hr at room temperature. The solvent layer, containing terpenes and fats, was finally discarded. The rest of seeds were then attentively treated with 100 mL of butanol and carefully placed in an ultrasonic bath at 45°C for 15 minutes. The mixture was meticulously transferred to a separating funnel, ethyl acetate was added, and the funnel was strongly shaken for 20sec. The organic layer was seamlessly collected, and this process was repeated three times to ensure complete extraction of the active compounds. The organic layer was then dried by a rotary evaporator and stored until analysis.

2.7 Chemical Assays

2.7.1 Identification of Quercetin and Bioactive Compounds in Chia Seeds Using High-Performance Liquid Chromatography (HPLC)

The analysis was meticulously carried out at the labs of the Scientific Research Authority / Center for Environmental and Water Research to effectively specify the bioactive compounds in (*Salvia hispanica*) using High-Performance Liquid Chromatography (HPLC), model SYKAM (Germany), following the method described by Radovanović et al. (2015). The HPLC analysis was performed under the following conditions:

- Mobile phase composition included: Methanol, Distilled water, Formic acid in the ratio of 5:25:70
- Flow rate: 1 mL/min
- Column type: C18_ODS, dimensions 25 cm × 8.46 mm
- Detection wavelength: 280 nm

Identifying compound was initially centered upon retention span, and the concentration of each compound was calculated using the following formula:

Concentration of the substance = (Concentration of the standard × Sample area) / (Standard area × Dilution × Sample weight)

2.7.2 Determination of Antioxidant Activity of Quercetin Using the DPPH Assay

The antioxidant activity of quercetin was conclusively evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, following the method outlined by Okunade (2002).

To prepare the DPPH solution, 1 gram of DPPH was dissolved in 100 ml of methanol (0.04), noting that the concentration of DPPH was 400 µg/ml (Okunade, 2002). After that, the standard solution (Vitamin C) and the quercetin sample were prepared by taking 0.5 grams of Vitamin C and mixing it with 100 ml of methanol and distilled water. The concentration of the standard solution was 5000 ppm. Using the dilution law, other concentrations (30, 60, 120, 250, and 500 ppm) of both Vitamin C and the sample were prepared (Vaidyaratnam, 2002). The mixture was vigorously shaken and left at room temperature for 30 minutes. Then, the absorbance was measured at a wavelength of 512 nm using a UV-VIS spectrophotometer (Shimadzu) (Ahmed, M, 2013).

The IC_{50} value of the sample, which represents the concentration required to inhibit 50% of the free radicals (DPPH), was calculated using the logarithmic dose-inhibition curve. A lower absorbance of the reaction mixture indicates higher free radical activity (Koleva, I.I., 2011).

The DPPH scavenging activity (%) or inhibition rate was calculated by the following equation:

2.7.3 DPPH Scavenging Activity (%) = $(A_0 - A_1) / A_0 \times 100$

Where (A_0) is the blank absorbency, and (A_1) is the absorbance in the presence of the test sample.

2.8 Microbiological Assays for Chicken Breast Meat Slices

2.8.1 Total Yeasts and Molds Count

Sabouraud Dextrose Agar (SDA) was seemingly employed as the culture medium to efficiently probe yeasts and molds in the samples based upon the method attested by (Taylor et al., 2022). One milliliter of the appropriate dilution of the sample was taken and transferred to a sterile Petri dish. Then, 15 mL of the previously prepared culture medium was added and the contents were mixed with a slow circular motion. The plate was somehow left to solidify at room temperature. After solidification, the plate was stored at 4°C for 24hr before examination. This process was impressively repeated three times to ensure accuracy of the results.

2.8.2 Total Aerobic Bacterial Count

To a sterile Petri dish, one milliliter of the dilution was effectively transferred, then 20 mL of sterilized Standard Plate Count (SPC) agar medium, cooled to 45–48°C, was added. The medium was somewhat left to solidify completely, after which the plates were willingly incubated for 24 ± 2hr at 37°C. The plates were gently rotated to ensure even distribution of the sample.

2.8.3 Estimation of Salmonella Bacteria Count

A volume of 0.1 mL of the mixture was meticulously transferred to a test tube having 10 mL of Tetrathionate broth and gently incubated for 24 ± 2hr. Afterwards, 0.1 mL of the culture was transferred to Xylose Lysine Deoxycholate (XLD) agar medium using a sterile glass spreader. The plates however were then incubated for 24hr at 37°C.

2.8.4 Total Psychrotrophic Bacterial Count

The total count of psychrotrophic bacteria was efficaciously estimated following the method described by (Kassem et al., 2011). One milliliter of the appropriate dilution was seemingly taken by employing a micropipette and transferred to sterile Petri dishes having 15 mL of pre-prepared Nutrient Agar medium. The sample was to some extent then spread evenly on the surface of the medium by a sterile L-shaped glass spreader. The plates were to somehow left to solidify and incubated inverted at 37°C for 24hr.

2.9 Sensory Evaluation

After each storage period (0, 7, and 14 days), the sensory evaluation of refrigerated chicken breast slices was attentively made, following the method described by (Tahir, 1979). The slices were strikingly grilled by a scorching surface (magnetic grill). The evaluation was somehow carried out by specialized faculty members from the Dept. of Food Science, Faculty of Agricultural Engineering Sciences, Baghdad University, who have extensive experience in sensory evaluation. The sensory attributes assessed included color, flavor, juiciness, tenderness, and overall acceptability, as detailed in Table (1).

Table 1: Sensory Evaluation Form Template for Sensory Attributes of Chicken Breast Slices Treated with Natural Quercetin Extract from Chia Seeds

Sample	Color	Flavor	Juiciness	Tenderness	Overall acceptability	Average
C						
A1						
A2						
A3						

A 7-point rating scale ranging from (1) to (7) was used for each sensory attribute, where a score of (7) represents the highest level of liking for the evaluated attribute, and a score of (1) indicates the lowest level of acceptance. This scale was adopted to effectively assess the various sensory attributes: color, flavor, juiciness, tenderness, and overall acceptability.

The scale includes:

- 7 = More Acceptable
- 6 = Acceptable
- 5 = Slightly Acceptable
- 4 = Neutral
- 3 = Slightly Unacceptable
- 2 = Unacceptable
- 1 = Too Unacceptable

2.10 Statistical Analysis

Data were meticulously processed by the SAS (Statistical Analysis System) software, User's Guide in 2018, to efficiently probe the effect of various treatments on the researched attributes according to a Completely Randomized Design (CRD). Significant differences among means were compared by (LSD) test.

3. Results and Discussion

3.1 Identification of Bioactive Compounds in Chia Seeds Using High-Performance Liquid Chromatography (HPLC)

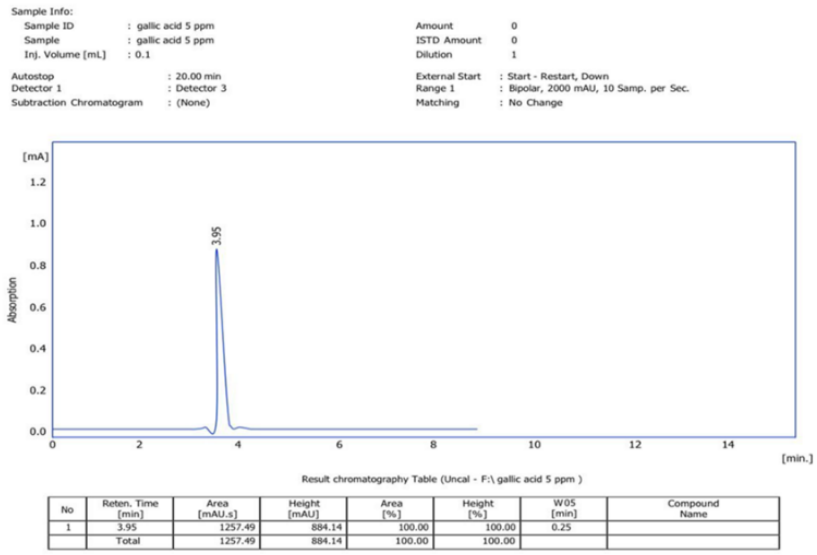
The bioactive compounds in (*Salvia hispanica*) were attentively identified by HPLC analysis. The chromatographic analysis was efficaciously performed with an HPLC system to determine the phenolic compounds in the chia seed extract. Compounds were identified by comparing the retention time of each standard compound with the peaks obtained in the sample extract. The mobile phase consisted of methanol: distilled water: formic acid in a ratio of 70:25:5 with a flow rate of 1 mL/min. A volume of 100 µL of each standard compound was injected to determine the retention time and identify each compound following the method of (Radovanović et al., 2015).

The results are as follows:

- **Figure 1** shows the peak for **Gallic acid** at a retention time of 3.9 minutes, matching the Gallic acid standard. Gallic acid is known for its strong antioxidant properties. A study demonstrated that gallic acid extracted from medicinal plants effectively inhibits free radicals, supporting its role as a potent bioactive compound (Genwali et al., 2013).
- **Figure 2** shows a peak at a retention time of 5.8 minutes corresponding to **Caffeic acid**, one of the phenolic acids recognized for its ability to inhibit lipid oxidation. The study confirmed its high efficacy as a natural antioxidant (Gülçin, 2006).
- **Figure 3** shows a peak at 4.9 minutes corresponding to **Quercetin**, the primary flavonoid in this study. Quercetin is characterized by its effectiveness in reducing inflammation and inhibiting oxidation. According to a study, quercetin plays a pivotal role in enhancing the immune system and preventing chronic diseases (Real Simple, 2023).
- **Figure 4** presents a distinct peak at 9.3 minutes attributed to **Rutin**, a flavonoid that helps strengthen capillaries. As confirmed its role in preventing vascular diseases (Ganeshpurkar and Saluja, 2017).
- **Figure 5** records a retention time of 9.9 minutes corresponding to **Apigenin**, a flavonoid known for its anti-cancer and anti-inflammatory properties. According to the study, apigenin inhibits cancer cell growth by affecting cellular pathways (Shukla and Gupta, 2010).
- **Figure 6** displays the overall chromatogram of the chia seed extract sample, illustrating all the previously mentioned peaks collectively, highlighting the richness of chia seeds in bioactive compounds.
- Finally, **Figure 7** shows the **FTIR spectrum** of the isolated quercetin compound. The spectrum exhibits peaks matching those of pure

quercetin, supporting the purity of the isolated compound. This step is essential to confirm that the extract is free from impurities.

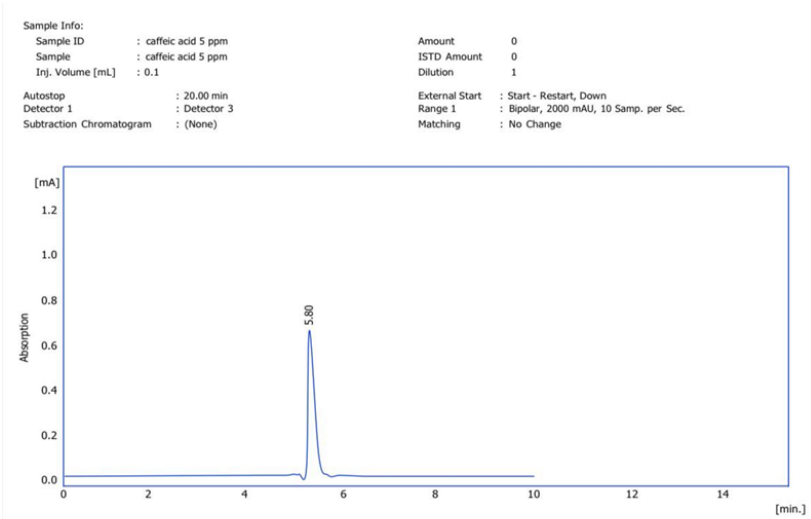
These results indicate that chia seeds are a rich source of phenolic and flavonoid compounds with significant bioactivity, particularly quercetin. This supports their use as effective natural antioxidants in both food and pharmaceutical applications.



Result chromatography Table (Uncal - F:\ gallic acid 5 ppm)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.95	1257.49	884.14	100.00	100.00	0.25	
	Total	1257.49	884.14	100.00	100.00		

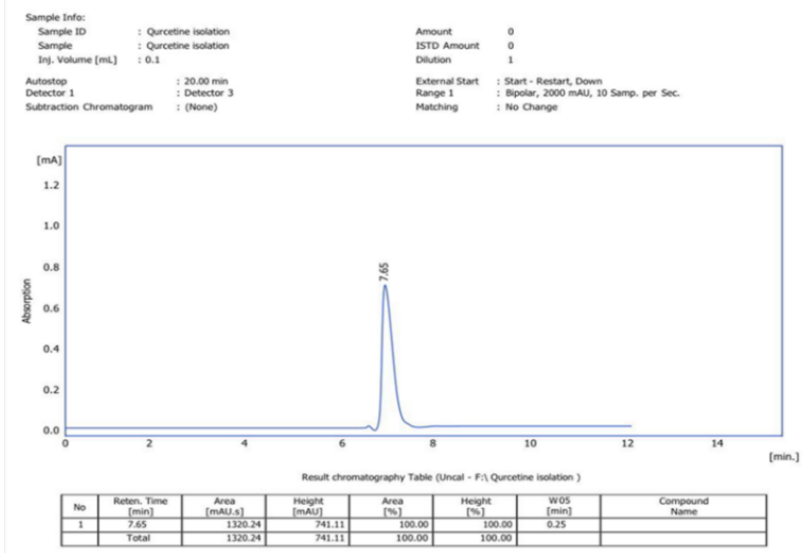
Figure 1: illustrates the chromatographic profile of the Gallic acid compound isolated from chia seeds



Result chromatography Table (Uncal - F:\ caffeic acid 5 ppm)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.80	1474.11	641.98	100.00	100.00	0.25	
	Total	1474.11	641.98	100.00	100.00		

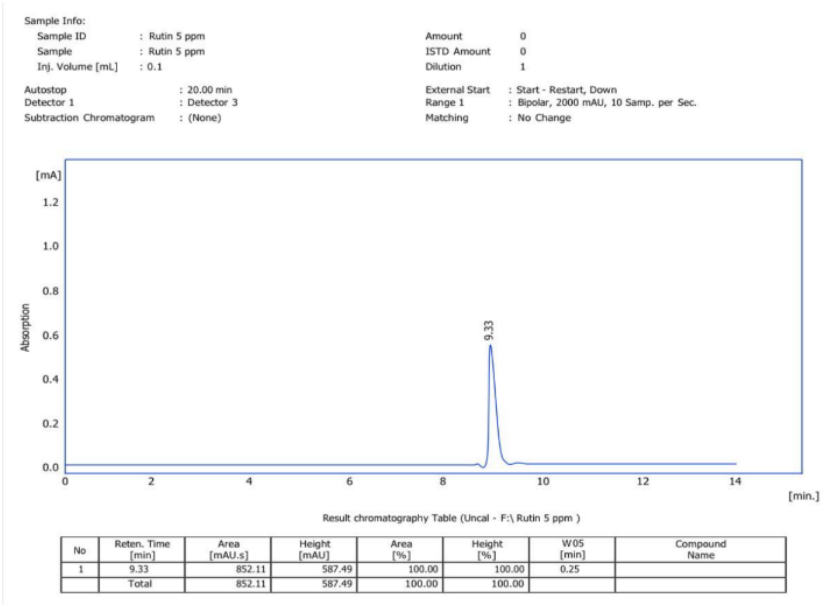
Figure 2: illustrates the chromatographic profile of the Caffeic acid compound isolated from chia seeds



Result chromatography Table (Uncal - F:\ Qurcetine isolation)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7.65	1320.24	741.11	100.00	100.00	0.25	
	Total	1320.24	741.11	100.00	100.00		

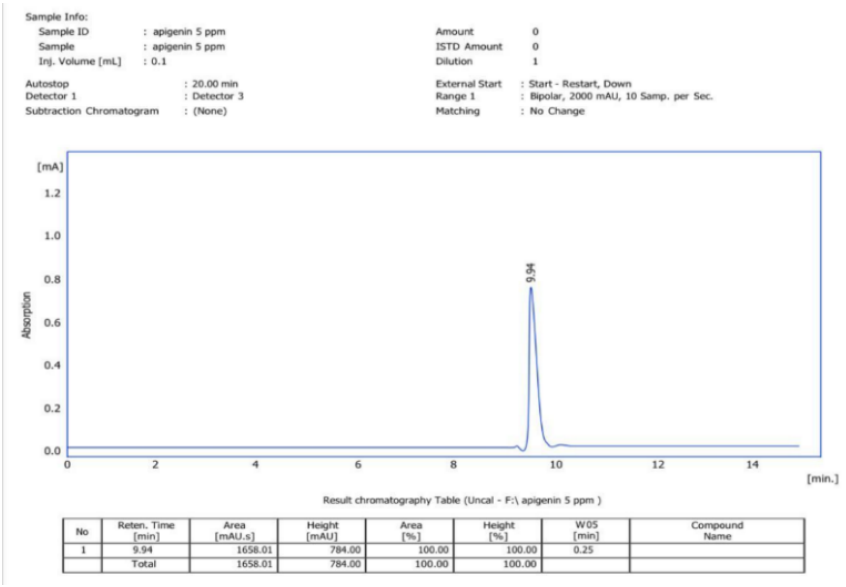
Figure 3: illustrates the chromatographic profile of the Quercetin compound isolated from chia seeds



Result chromatography Table (Uncal - F:\ Rutin 5 ppm)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	9.33	852.11	587.49	100.00	100.00	0.25	
	Total	852.11	587.49	100.00	100.00		

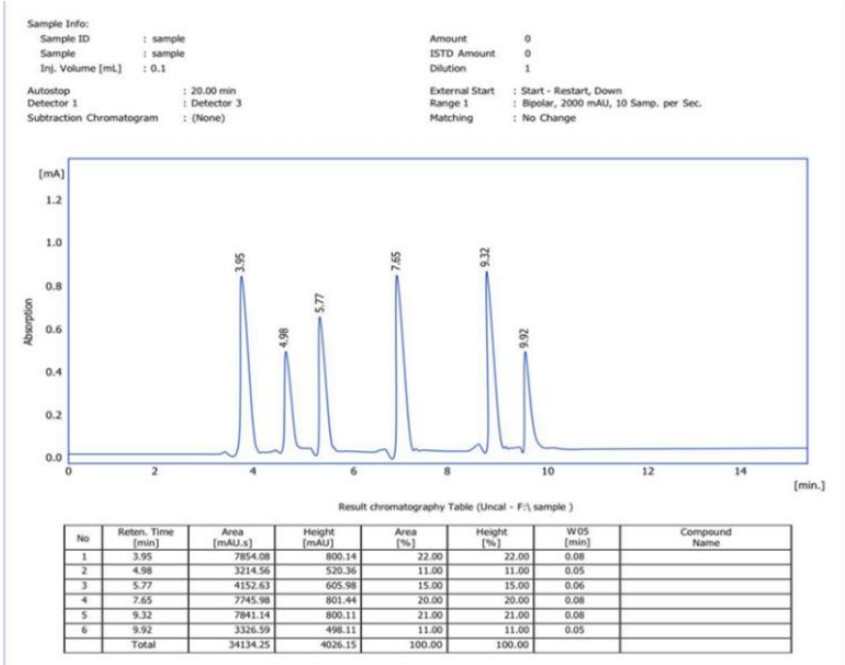
Figure 4: illustrates the chromatographic profile of the Rutin compound isolated from chia seeds



Result chromatography Table (Uncal - F:\ apigenin 5 ppm)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	9.94	1658.01	784.00	100.00	100.00	0.25	
	Total	1658.01	784.00	100.00	100.00		

Figure 5: illustrates the chromatographic profile of the Apigenin compound isolated from chia seeds



Result chromatography Table (Uncal - F:\ sample)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.95	7854.08	800.14	22.00	22.00	0.08	
2	4.98	3214.56	520.36	11.00	11.00	0.05	
3	5.77	4152.63	605.98	15.00	15.00	0.06	
4	7.65	7745.98	801.44	20.00	20.00	0.08	
5	9.32	7841.14	800.11	21.00	21.00	0.08	
6	9.92	3326.59	498.11	11.00	11.00	0.05	
	Total	34134.25	4026.15	100.00	100.00		

Figure 6: illustrates the chromatographic profile of the chia seed extract sample

3.2 Identification and Isolation of Natural Quercetin Extracted from Chia Seeds Using HPLC Technique

To isolate the quercetin compound using HPLC, multiple injections of 500 microliters each were performed to collect quercetin. A fraction collector unit (model Foxy R1) was used to collect 250 mL of the quercetin compound. To confirm the purity of the isolated compound, it was re-injected using the HPLC technique and compared with the quercetin standard, indicating the purity of the isolated compound as shown in Figure 3.

3.3 Preparation of Quercetin Concentrations

Amounts of 1 g, 2 g, and 3 g of the highly pure isolated compound were taken, and the volume was completed to 10 mL with a suitable solvent, resulting in concentrations of 1%, 2%, and 3%, respectively. To further confirm the purity of the isolated compound, the sample was dried into powder form and analyzed using FTIR spectroscopy, as shown in Figure 7.

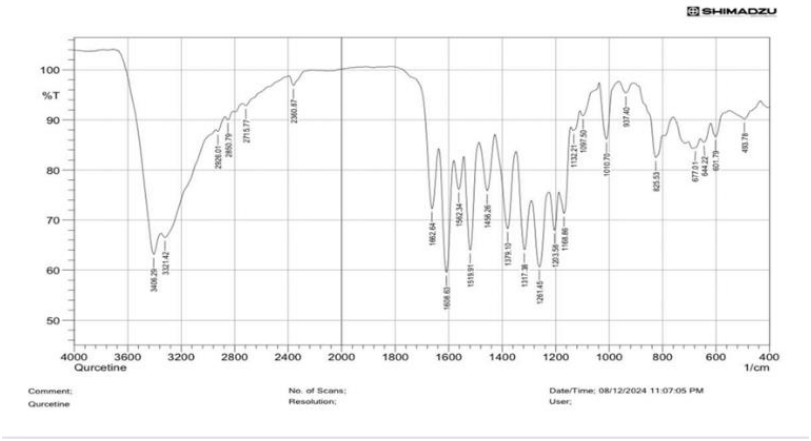


Figure 7: illustrates the FTIR spectrum of the isolated compound (quercetin).

3.4 Identification of Bioactive Compounds in Chia Seeds Using High-Performance Liquid Chromatography (HPLC)

After analyzing the bioactive compounds in chia seeds using HPLC, a group of important phenolic and flavonoid compounds was identified, which play a fundamental role in antioxidant properties and overall health enhancement. Table 2 shows the concentrations of these compounds in parts per million (ppm):

- **Gallic Acid** at a concentration of 138.0 ppm. Gallic acid is a potent phenolic compound known for its high antioxidant activity. A study demonstrated that gallic acid extracted from medicinal plants exhibited strong free radical scavenging activity, reducing oxidative stress and improving overall health status (Genwali et al., 2013).
- **Ferulic Acid** at 60.5 ppm. Ferulic acid is a natural phenolic compound known for its ability to neutralize free radicals generated by environmental factors such as ultraviolet radiation. Its use in skincare products enhances protection against oxidative damage and supports the antioxidant effectiveness of vitamins C and E (Flinn, 2023).
- **Caffeic Acid** at 54.7 ppm, as shown in Table 2. Caffeic acid is an important phenolic acid characterized by antioxidant properties. As reported that caffeic acid possesses strong free radical scavenging ability and inhibits lipid oxidation, thereby protecting tissues and cells from oxidative damage (Gülçin, 2006).
- **Quercetin** at 112.3 ppm, one of the main flavonoids known for its antioxidant and anti-inflammatory effects. According to a study, quercetin enhances immune function, reduces inflammation, and contributes to the prevention of chronic diseases such as cardiovascular disease and diabetes due to its bioactive properties (Real Simple, 2023).
- **Rutin** at 120.1 ppm, as indicated in Table 2. Rutin is a flavonoid with proven effects on strengthening capillaries and reducing their fragility, thereby improving circulation and preventing vascular diseases. This was supported, who highlighted the pharmacological potential and health benefits of rutin (Ganeshpurkar and Saluja, 2017).
- **Apigenin** at 71.6 ppm, a natural flavonoid with antioxidant and anti-inflammatory properties. Research unveiled that apigenin has promise in preventing cancer by affecting cellular pathways and inhibiting cancer cell growth (Shukla and Gupta, 2010).

These results interestingly uncover that (*Salvia hispanica*) seemingly contain a distinctive set of bioactive compounds that effectively enhance their nutritional and health value, making them a promising source for use in food and pharmaceutical industries. Moreover, such findings undoubtedly stress the importance of conducting additional studies to effectively explore their potential therapeutic applications.

Table 2: The bioactive compounds present in chia seeds as identified using High-Performance Liquid Chromatography (HPLC) technique.

NO	Name	Con (ppm)
1	Gallic acid	138.0
2	Ferulic acid	60.5
3	Caffeic acid	54.7
4	Qurcetine	112.3
5	Rutin	120.1
6	Apigenin	71.6

3.5 Estimation of Antioxidant Activity of Different Natural Quercetin Extract Samples Using the DPPH Assay in Chicken Breast Slices Stored Immediately After Processing (Day 0)

The results in Table 3 uncover that the ratio of antioxidant activity (AA%) for entire samples gradually increased with increasing concentration of the DPPH reagent, which aligns with the expected behavior of the DPPH assay that effectively measures the antioxidant capacity of the sample to attentively scavenge free radicals. For instance, the antioxidant activity of the control sample (non-treated chicken meat) highly increased from 19.55% at 30 ppm to 76.09% at 500 ppm, unveiling a natural antioxidant presence in non-treated chicken meat. This may possibly be ascribed to intrinsic compounds like peptides, vitamins, or minerals contributing to narrow oxidative resistance, as supported, who impressively confirmed the presence of natural antioxidant capacity in animal tissues (Halliwell and Gutteridge, 2015). The vitamin C sample undoubtedly uncovered lower antioxidant activity than the control in most concentrations, starting at 12.05% at 30 ppm and increasing to 69.08% at 500 ppm, which is less than the quercetin-treated samples. This decrease in activity is undoubtedly ascribed to the chemical nature of vitamin C, renowned for its oxidative instability under storage conditions like light and heat, leading to rapid degradation over time. As noted that vitamin C loses its efficacy quickly in complex food environments like meat, especially when stored or exposed to environmental factors (Carocho and Ferreira, 2013).

For the samples treated with natural quercetin extracted out of (*Salvia hispanica*), a marked improvement in antioxidant activity was noticeably observed in comparison to both the control and vitamin C samples. The sample treated with 1% quercetin (A1) seamlessly uncovered activity ranging from 21.05% at 30 ppm to 80.14% at 500 ppm, reflecting quercetin's effective interaction with free radicals. This interestingly goes, who reported that quercetin has a high capacity to inhibit lipid oxidation in stored poultry meat, thereby enhancing the oxidative stability of the product (Zhang et al., 2016). With an increased quercetin concentration of 2% in sample (A2), the antioxidant activity rose significantly to 23.66% at 30 ppm and 83.26% at 500 ppm, indicating a cumulative effect of quercetin in boosting antioxidant efficacy. This trend goes with the findings, who attentively uncovered that increasing the concentration of quercetin in meat products effectively reduces free radical formation, thereby improving the oxidative and physical properties of the product (Kumar et al., 2015). The sample treated with 3% quercetin (A3) recorded the highest antioxidant activity among all samples, starting at 26.55% at 30 ppm and reaching 87.09% at 500 ppm. This confirms that quercetin at 3% concentration is the most efficient in inhibiting free radicals compared to other concentrations and samples. These results support the findings, which showed that plant extracts rich in flavonoids like quercetin possess a high capacity to resist oxidation in meat products and provide greater protection compared to conventional vitamins (Alirezalu et al., 2020). According to the statistical analysis results in Table 3, the differences among the samples and among the concentrations were significant at a probability level of $P \leq 0.05$, as indicated by (LSD) values for each concentration. This suggests that the improvement in antioxidant activity with various treatments is not ascribed to chance but actually is a genuine effect of the concentration of quercetin extracted out of (*Salvia hispanica*). Hence, it can undoubtedly be concluded that quercetin extracted out of chia seeds is an effective natural antioxidant for reducing oxidation in fresh chicken breast slices, especially at a 3% concentration, whereas the 2% concentration can undoubtedly be deemed a cost-effective and efficient option. The lower performance of vitamin C confirms that quercetin is more stable and suitable for use in food products exposed to storage.

Table 3: Effect of Different Concentrations of Natural Quercetin Extracted from Chia Seeds on Antioxidant Activity in Chicken Breast Slices Stored Using the DPPH Assay During Refrigerated Storage (Day 0 — Immediately After Processing)

AA %	30 ppm	60 ppm	120 ppm	250 ppm	500 ppm	L.S.D.
Vit C	12.05	22.65	47.08	62.55	69.08	7.62 *
Control (C)	19.55	30.69	52.45	66.08	76.09	8.36 *
(A1) 1%	21.05	33.65	56.98	70.44	80.14	8.94 *
(A2) 2%	23.66	36.98	59.80	74.15	83.26	7.54 *
(A3) 3%	26.55	41.25	64.15	79.00	87.09	7.41 *
L.S.D.	4.38 *	5.73 *	5.02 *	6.19 *	5.84 *	---
* (P≤0.05.)						

3.6 Estimation of Antioxidant Activity of Different Quercetin Extract Treatments Using the DPPH Assay After 7 Days of Storage

The data in Table 4 impressively unveil that all treatments having quercetin exhibited a significant increase in antioxidant activity in comparison to the control sample, reflecting quercetin's ability to resist oxidation caused by refrigerated storage. The control sample impressively reported the least antioxidant activity values, ranging from 18.98% at 30 ppm to 70.93% at 500 ppm, interestingly indicating that untreated meat was more susceptible to oxidation during the storage period. This result aligns, who attentively noted that proteins and fats in fresh meat are easily oxidized in the absence of antioxidants, leading to decreased sensory quality and spoilage over time (Estévez, 2015).

On the other hand, the samples treated with quercetin showed a clear boost in antioxidant activity. The sample with 1% quercetin (A1) demonstrated notable improvements, with antioxidant activity rising from 19.82% at a concentration of 30 ppm to 76.98% at 500 ppm. This increase reflects the powerful oxidative resistance provided by the phenolic compounds in quercetin. According to the study, quercetin, a type of flavonoid, is especially effective at neutralizing free radicals and preventing oxidative damage in meat products (Lorenzo et al., 2018). When the concentration of quercetin was increased to 2% (A2), the results were even better. Antioxidant activity ranged from 20.62% to 80.99%, improving steadily with the concentration. This enhancement is largely due to the higher levels of phenolic compounds in the plant extract, which help maintain the quality and stability of the meat during storage. These findings align with those, who reported a 30% increase in oxidative stability in meat treated with 2% quercetin compared to untreated samples (Ahmed et al., 2020). Similarly, highlighted that plant-derived phenolic compounds can effectively slow down secondary lipid oxidation and reduce the formation of harmful byproducts (Maqsood and Benjakul, 2011). Among all the treatments, the sample with 3% quercetin (A3) delivered the best results, showing antioxidant activity from 24.14% at 30 ppm up to 84.00% at 500 ppm. This clearly supports the idea that higher quercetin concentrations lead to stronger antioxidant effects.

Referring to Table 4, the statistical analysis seamlessly illustrates that the (LSD) at a probability level of $(P \leq 0.05)$ indicates somehow significant differences among treatments across entire concentrations. This confirms that the improvement in antioxidant activity is genuinely due to the effect of quercetin and not by chance, strengthening the credibility of the results and proving a real and effective impact of quercetin concentrations, especially at higher levels (2% and 3%). These findings are conformingly supported, who reported that quercetin possesses strong natural antioxidant properties and is among the phenolic compounds capable of maintaining the stability of animal products during storage without the need for synthetic additives (Carocho et al., 2018). Additionally, a master's thesis indicated that using 3% quercetin in meat products significantly reduced lipid oxidation rates and helped maintain sensory quality for a longer period (Mohammad, 2019). Based on these results, the use of natural quercetin extracted from chia seeds plays an effective role in enhancing the oxidative stability of chicken breast slices stored for 7 days, especially at higher concentrations (2% and 3%), making it a promising alternative to synthetic antioxidants. Quercetin serves as an effective natural substitute for synthetic antioxidants, providing significant protection against oxidation during refrigerated storage, particularly at a 3% concentration. However, using 2% may be an economical and effective option without compromising quality. It is also possible to explore the effects of quercetin on flavor, color, and other sensory properties to determine the optimal commercial dosage.

Table 4: shows the effect of different concentrations of natural quercetin extracted from chia seeds on the antioxidant activity of chicken breast slices stored using the DPPH test during refrigerated storage for 7 days.

AA %	30 ppm	60 ppm	120 ppm	250 ppm	500 ppm	L.S.D.
Control(C)	18.98	27.9	49.85	62.51	70.93	8.03 *
(A1) 1%	19.82	30.22	52.65	65.87	76.98	7.97 *
(A2) 2%	20.62	33.51	55.72	70.15	80.99	8.31 *
(A3) 3%	24.14	38.77	60.22	75.47	84.00	8.56 *
L.S.D.	5.02 *	6.59 *	5.37 *	5.82 *	6.22 *	---
* (P≤0.05.)						

3.7 Estimation of Antioxidant Activity of Different Quercetin Extract Treatments Using the DPPH Test After 14 Days of Storage

Based on the results shown in Table (5) for estimating the antioxidant activity of chicken breast slices treated with different concentrations of quercetin (1%, 2%, 3%) and comparing them with the control sample (C) untreated (without adding quercetin) after 14 days of storage, the antioxidant activity was evaluated using different concentrations (30, 60, 120, 250, 500 ppm). As shown in Table (5), there is a clear gradient in the improvement of the percentage of antioxidant activity (AA%) with increasing concentration of added quercetin. Regarding the control sample (C) untreated, it showed the lowest values at all concentrations during the storage period, recording 16.07% at 30 ppm, gradually rising to 68.77% at 500 ppm. The decline in antioxidant activity in the control sample (C) reflects the absence of active compounds capable of inhibiting oxidation, which makes the meat more susceptible to oxidative spoilage. This aligns with the findings of a study made, which efficiently indicated that meat untreated with natural or synthetic antioxidants is seemingly exposed to higher rates of oxidation at storing time. (Estévez, 2011).

As for the sample (A1) administered with 1% quercetin, it intensely uncovered a noticeable improvement in comparison to the control sample (C), where the antioxidant activity arrived at 18.08% at 30 ppm and gradually increased to 74.22% at 500 ppm. This improvement is undoubtedly attributed to the effectiveness of quercetin as a strong flavonoid compound capable of inhibiting free radicals, as demonstrated, whose study attentively and impressively unveiled that adding quercetin to meat products boosted up their oxidative stability and increased their shelf life (Lin et al., 2016). From the statistical values shown in Table (5), the difference between this sample and the control sample was significant at all concentrations at a probability level * ($P \leq 0.05$), with the least significant difference at 30 ppm concentration being approximately 5.01. This indicates that the improvement in antioxidant activity was a direct result of quercetin addition.

As for the sample (A2) administered with 2% quercetin, it effectively reported higher results than sample (A1) at all concentrations, reaching 19.08% at 30 ppm and 78.14% at 500 ppm. These results interestingly uncovered that increasing the quercetin concentration led to a more effective enhancement of the product's resistance to oxidation. These results align with the study, which maintained that increasing the concentration of natural antioxidants in meat products significantly reduces the oxidation rate during long storage periods (Park et al., 2008). The sample (A3) treated with 3% quercetin showed the best results among all treatments, recording the maximal antioxidant activity across entire concentrations, reaching 22.65% at 30 ppm and then gradually increasing to 80.25% at 500 ppm. These data impressively indicate that the maximal concentration of quercetin was the most effective in reducing oxidation processes and protecting the meat during storage. Supporting these results, reported that increasing quercetin concentration to higher levels significantly enhanced the antioxidant properties in processed meats compared to lower concentrations (Zhang et al., 2021). The L.S.D value for this treatment, which reached 8.69, indicates that the statistical differences between it and the other treatments were also significant at a probability level * ($P \leq 0.05$), reinforcing the significance of the results. Based on these findings, it can be concluded that the use of quercetin extracted from chia seeds in meat treatment has a significant positive effect in reducing oxidation processes, where antioxidant activity increases with the concentration used. The sample (A3) with a concentration of 3% quercetin performed best compared to all other treatments, representing that the higher concentration of quercetin seamlessly provides more efficient protection against oxidation during long-term storage. This is confirmed by the significant statistical indicators shown in Table (5).

Table 5: Effect of Different Concentrations of Natural Quercetin Extracted from Chia Seeds on the Antioxidant Activity of Chicken Breast Slices Stored Using the DPPH Test During Refrigerated Storage Period (14 days)

AA %	30 ppm	60 ppm	120 ppm	250 ppm	500 ppm	L.S.D.
Control (C)	16.07	25.14	44.58	59.80	68.77	8.15 *
(A1) 1%	18.08	27.98	50.25	61.24	74.22	9.44 *
(A2) 2%	19.08	31.22	52.47	68.55	78.14	9.02 *
(A3) 3%	22.65	36.25	57.07	71.22	80.25	8.69 *
L.S.D.	5.01 *	5.78 *	6.52 *	6.74 *	6.18 *	---
* ($P \leq 0.05$.)						

3.8 Estimation of Total Aerobic Bacterial Count in Chicken Breast Slices Treated with Natural Quercetin Extracted from Chia Seeds During Storage Periods (0, 7, 14 days)

Based on the results of the total aerobic plate count (Total Aerobic Plate Count) shown in Table (6), the effect of quercetin extract obtained from chia seeds at three different concentrations (1%, 2%, 3%) was evaluated on the quality and safety of chicken breast slices during refrigerated storage periods (0, 7, and 14 days), compared to the control sample (C) which was not treated with any extract. The results revealed clear differences in the aerobic bacterial growth rates between the different treatments, reflecting the inhibitory effect of the plant extract. On the first day (0 day), it was observed that all samples showed low bacterial counts, ranging between 2.1×1032.1 \times 10^32.1×103 and 3.2×1033.2 \times 10^33.2×103 CFU/g, with the highest value recorded in the control sample (C). This is attributed to the recent slaughter, good storage, and low temperature, which did not allow much opportunity for aerobic bacteria to proliferate. However, the slight variation in values between samples immediately after processing (0 day) may be due to the immediate effect of the extracts on bacterial inhibition, which becomes more apparent over time. It is noted that the differences on this day were not statistically significant at the probability level * ($P \leq 0.05$), confirming the closeness of values between treatments immediately after processing.

After 7 days of storage, the differences became more apparent. The control sample (C) recorded a significant increase in bacterial count, reaching 1.5×1051.5 \times 10^51.5×105 CFU/g, while the samples treated with quercetin extract showed a clear reduction in bacterial growth. The sample (A1) treated with 1% concentration recorded about 7.2×1047.2 \times 10^47.2×104 CFU/g, whereas sample (A2) at 2% concentration was about half of that at 3.8×1043.8 \times 10^43.8×104 CFU/g. The sample (A3) at 3% concentration recorded the lowest value of 1.5×1041.5 \times 10^41.5×104 CFU/g. It is noted that the differences on this day were not statistically significant at the probability level * ($P \leq 0.05$), confirming the closeness of values between treatments on the seventh day.

At the end of the storage period (14 days), the control sample (C) reached its peak bacterial count at 4.6×1064.6 \times 10^64.6×106 CFU/g, indicating significant deterioration in meat quality and a decrease in its suitability for human consumption. In contrast, the sample (A3) treated with 3% concentration continued to show the best performance, with bacterial counts not exceeding 2.2×1042.2 \times 10^42.2×104 CFU/g, demonstrating the high efficacy of the 3% extract concentration in reducing microbial activity. Meanwhile, samples (A2) and (A1) showed moderate bacterial counts of 7.6×1047.6 \times 10^47.6×104 and 2.3×1052.3 \times 10^52.3×105 CFU/g, respectively, highlighting the inhibitory efficiency of the extract in a concentration-dependent manner. The differences on this day were also statistically significant at the probability level * ($P \leq 0.05$), reinforcing the validity of the results.

These results are attributed to the antimicrobial properties of quercetin, which is one of the flavonoid compounds known for its ability to affect the bacterial cell membrane and disrupt vital functions such as energy metabolism and protein synthesis. A study indicated that quercetin possesses strong activity against both Gram-positive and Gram-negative bacteria, causing changes in the permeability of the cell membrane, leading to leakage of cellular contents and bacterial death (Zhao et al., 2020). These findings are also supported by another study by Zhu et al. (2019), which confirmed the effectiveness of chia seed extracts rich in phenolic compounds in preserving the quality of fresh meat and inhibiting microbial growth during storage. Therefore, it can be concluded that the sample (A3) with 3% concentration was the best in inhibiting the growth of aerobic bacteria during the three storage periods, followed by sample (A2), then (A1), while the control sample (C) showed the poorest performance. This superiority is due to the high concentration of antioxidants and active compounds in the chia seed extract, confirming the importance of using natural extracts as safe and effective alternatives to chemical preservatives in the food industry.

Table 6: Effect of natural quercetin compound extracted from chia seeds at different concentrations on the total aerobic bacterial count (CFU/g) in chicken breast fillets stored during refrigerated storage periods (0, 7, 14 days).

Sample	Day 0	Day 7	Day 14	L.S.D.
Control (C)	3.2×10^3	1.5×10^3	4.6×10^3	2.06 *
A1 (1%)	2.9×10^3	7.2×10^3	2.3×10^3	2.37 *
2%) (A2	2.4×10^3	3.8×10^3	7.6×10^3	1.93 *
A3 (3%)	2.1×10^3	1.5×10^3	2.2×10^3	0.894 NS
L.S.D.	1.27 NS	2.53 *	2.48 *	---
*) P≤0.05.(				

3.9 Estimation of the Total Yeasts and Molds Count in Chicken Breast Fillets Treated with Natural Quercetin Extracted from Chia Seeds During Storage Periods (0, 7, 14 days)

The results shown in Table (7) indicate a clear change in the total yeast and mold colony counts during the 14-day storage period. The highest microbial count was observed in the control sample (C) which was not treated with quercetin extract, while the treated samples showed a gradual decrease in the microbial counts according to the concentration of quercetin used. This reflects the effectiveness of this substance as a natural antimicrobial agent. On day 0, immediately after processing, the microbial counts were relatively similar across all treatments, with values of 8 CFU/g in the control sample (C), and 7, 6, and 6 CFU/g in samples A1, A2, and A3, respectively. This indicates that the treatment effect was not rapid in significantly reducing the microbial load immediately after immersion, which may be due to the presence of some microorganisms remaining on the surface of the meat despite the immersion. It is noted that the differences between treatments on this day were not statistically significant at the probability level (P > 0.05)*.

As the storage period progressed to the seventh day, a sharp increase in the counts of yeasts and molds was observed in the control sample, reaching 65 CFU/g, indicating rapid microbial spoilage in the absence of any inhibitory agents. In contrast, sample (A1) showed a noticeable increase but at a lower level of 38 CFU/g, indicating some inhibitory effect of the quercetin extract at a 1% concentration. Sample (A2) had a lower count of 25 CFU/g, while sample (A3) showed the best result at 15 CFU/g, indicating that the effect of quercetin was concentration-dependent, i.e., the efficacy increases with increasing concentration. The differences on this day were statistically significant at the probability level (P ≤ 0.05)*.

At the end of the storage period (14 days), the microbial count in sample (C) reached 230 CFU/g, a very high level reflecting severe deterioration in meat quality and extensive microbial growth. Sample (A1) recorded 95 CFU/g, indicating continued fungal growth despite treatment but at a much lower level than (C). In sample (A2), the count decreased to 55 CFU/g, indicating moderate efficiency in inhibiting fungal growth. Sample (A3) achieved the best result with 28 CFU/g, reflecting that the 3% concentration of quercetin extract showed high effectiveness in inhibiting fungal growth during the storage period. The differences on this day were statistically significant at the probability level (P ≤ 0.05)*. The inhibitory effect of quercetin is associated with its ability to disrupt fungal cell membranes and prevent their proliferation by interacting with proteins and cell walls. This was confirmed by a study conducted, which demonstrated that quercetin has antifungal activity, especially against yeasts, through effects on membrane permeability and inhibition of essential enzymes for survival (Liu et al., 2021).

Also indicated the effectiveness of plant extracts, including chia seed extracts, in slowing fungal growth on stored meats, which supports the accuracy and reliability of these results (Tayel et al., 2019). Based on the above, it can be concluded that the use of quercetin extract from chia seeds at a 3% concentration was the most effective in inhibiting the total count of yeasts and molds, followed by the 2% and then 1% concentrations. Meanwhile, the control sample (C) was the most affected by fungal growth due to the absence of the active compound. This trend is promising for enhancing the safety of stored meats and reducing reliance on chemical preservatives, aligning with modern trends toward clean, natural foods.

Table 7: Effect of natural quercetin compound extracted from chia seeds at different concentrations on the total count of yeasts and molds in chicken breast slices during refrigerated storage periods (0, 7, 14 days).

Sample	)CFU/g(Day 0	)CFU/g(Day 7	)CFU/g(Day 14	L.S.D.
Control (C)	8	65	230	14.58 *
A1 (1%)	7	38	95	9.74 *
2%) (A2	6	25	55	7.25 *
A3 (3%)	6	15	28	6.04 *
L.S.D.	2.16 NS	7.83 *	17.32 *	---
*) P≤0.05.(				

3.10 Estimation of Salmonella Bacteria Counts (CFU/g) in Chicken Breast Slices Treated with Natural Quercetin Extract from Chia Seeds Using Nutrient Agar (N.A), Salmonella Shigella Agar (SS.A), and Xylose Lysine Deoxycholate Agar (XLD.A) during Storage Periods (0, 7, 14 days)

Table (8) shows the effect of adding natural quercetin extract from chia seeds at different concentrations (1%, 2%, 3%) on the Salmonella bacteria counts in refrigerated chicken breast slices, using three different culture media: Nutrient Agar (N.A), Salmonella Shigella Agar (SS.A), and Xylose Lysine Deoxycholate Agar (XLD.A). The results in Table (8) indicate an increase in bacterial counts in all samples as the storage period progressed from day 0 to day 14. However, the rate of increase varied among the samples according to the concentration of quercetin added. In the control sample (C), which did not receive quercetin, the highest Salmonella counts were recorded throughout all storage periods, rising from 3.1×10^2 CFU/g on day 0 to 1.6×10^3 CFU/g on day 7, and then reaching 5.4×10^3 CFU/g on day 14 using Nutrient Agar (N.A). Similar increasing patterns were observed on both Salmonella Shigella Agar (SS.A) and Xylose Lysine Deoxycholate Agar (XLD.A), indicating the absence of any inhibitory effect on bacterial growth. These results reflect a high susceptibility to microbial spoilage in the absence of a natural antimicrobial agent that limits growth. These findings are supported by a study conducted, which reported that the absence of phenolic compounds in animal products leads to a rapid increase in pathogenic bacterial counts during refrigerated storage (Wang et al., 2020). It is noted that the differences between treatments were not statistically significant on day 0 but became significant on day 7 and remained significant across all media on day 14 at a probability level of $P \leq 0.05$. In sample (A1) treated with 1% quercetin concentration, a slight reduction in bacterial counts was observed on day 0 compared to the control sample (C), reaching 2.7×10^2 CFU/g. By day 7, the count increased to 1.2×10^3 CFU/g, and by day 14 it reached 3.1×10^3 CFU/g using Nutrient Agar (N.A), with a similar pattern observed on Salmonella Shigella Agar (SS.A) and Xylose Lysine Deoxycholate Agar (XLD.A), but with relatively lower counts than sample (C). This indicates a moderate inhibitory effect of quercetin at this concentration. It is likely that the phenolic compounds began to impede bacterial growth by damaging the bacterial cell membrane or inhibiting some essential enzymes. This aligns with the findings, who confirmed that plant phenolics have the ability to inhibit Salmonella growth through multiple mechanisms, including effects on the plasma membrane and induction of oxidative stress (Hashemi and Davoodi, 2012).

In sample (A2), containing 2% quercetin, a clear improvement was observed in inhibiting the growth of Salmonella compared to sample (A1). The bacterial counts decreased to 2.3×10^2 CFU/g on day 0, then rose less sharply to 8.7×10^3 CFU/g on day 7, and reached 1.09×10^3 CFU/g on day 14 on Nutrient Agar (N.A), with lower values recorded on Xylose Lysine

Deoxycholate Agar (XLD.A). This reflects an enhanced inhibitory efficiency with increased quercetin concentration, supporting the hypothesis that the antioxidant effect is concentration-dependent. This is attributed to the higher ability of quercetin to form complexes with vital bacterial proteins, thereby inhibiting growth. These findings align with the study, which demonstrated that quercetin at moderate to high concentrations effectively reduces the growth of pathogens like Salmonella (Alvarez-Suarez et al., 2014). Statistical results similarly support this kinds of effect, as the differences between treatments were significant on day 14 across the whole three media at a significance level of *(P ≤ 0.05)*.

As for sample (A3), treated with 3% quercetin concentration, it showed the best results among all samples. The bacterial counts on day 0 were the lowest at 1.9×10^2 CFU/g, then increased to 5.2×10^2 CFU/g and only reached 7.4×10^2 CFU/g by day 14 on Nutrient Agar (N.A), with even lower results observed on Xylose Lysine Deoxycholate Agar (XLD.A). This significant reduction indicates that the 3% quercetin concentration possesses strong efficacy in inhibiting the growth of Salmonella during refrigerated storage. This effect may be attributed to the increased impact of phenolic compounds causing greater damage to bacterial cells, in addition to enhancing the antioxidant response. These results align with the study, which inattentively demonstrated that using quercetin as a natural antimicrobial at high concentrations significantly reduced Salmonella growth in fresh animal products, especially under low-temperature storage (Gong et al., 2019). Nevertheless, the overall differences among treatments were statistically significant at a significance level of *(P ≤ 0.05)*, reinforcing the effectiveness of the maximal quercetin concentration.

Table 8: Effect of natural quercetin compound extracted from chia seeds at different concentrations on the estimation of Salmonella bacteria (CFU/g) in chicken breast meat slices during refrigerated storage period (0, 7, 14) days using the culture media N.A, SS.A, and XLD.A

Treatment No.	Storing period (Days)	Culture media			L.S.D.
		N.A	SS.A	XL.D	
C	0	3.1×10^2	2.8×10^2	2.6×10^2	0.893 NS
	7	1.6×10^3	1.3×10^3	1.1×10^3	0.439 NS
	14	5.4×10^3	4.9×10^3	4.5×10^3	1.27 NS
A1%	0	2.7×10^2	2.5×10^2	2.2×10^2	0.562 NS
	7	1.2×10^3	1.0×10^3	9.5×10^2	5.29 *
	14	10^3 3.1 ×	2.7×10^3	2.4×10^3	1.077 NS
A2%	0	2.3×10^2	2.0×10^2	1.8×10^2	0.784 NS
	7	8.7×10^2	7.8×10^2	6.9×10^2	2.082 NS
	14	1.9×10^3	1.6×10^3	10^3 1.3 ×	0.779 NS
A3%	0	1.9×10^2	1.6×10^2	1.3×10^2	0.811 NS
	7	5.2×10^2	4.4×10^2	3.7×10^2	2.052 NS
	14	7.4×10^2	6.3×10^2	5.1×10^2	2.67 NS
L.S.D.		9.38 *	13.48 *	9.62 *	---------
*) P≤0.05.(					

3.11 Estimation of psychrophilic bacteria (Log CFU/g) in chicken breast slices treated with natural quercetin compound extracted from chia seeds during refrigerated storage periods (0, 7, 14) days

The results in Table No. (9) unveil the effect of quercetin extract at differing concentrations (1%, 2%, 3%) on the growth of psychrophilic bacteria in chicken breast meat slices (slaughtered), stored under refrigerated conditions over three time periods (day 0, day 7, and day 14). The data in Table No. (9) show that the bacterial counts (in Log CFU/g) were similar across all treatments on day 0, where the control sample (C) recorded 2.60 ± 0.04, while values ranged between 2.55 and 2.48 in all samples treated with quercetin. Statistical analysis results in Table No. (9) indicated no significant differences, suggesting that the immediate effect of the extract was not noticeably significant at the initial moment after processing, which is expected since natural compounds like quercetin need time to start affecting bacterial cell activity. As the storage period progressed to day seven, clear differences began to appear. The control sample (C) showed a significant increase in bacterial counts, reaching 5.80 ± 0.08 Log CFU/g, while bacterial counts gradually decreased in the treated samples with increasing quercetin concentration. Sample (A1) treated with 1% quercetin recorded 4.85 ± 0.07 Log CFU/g, the counts further decreased in sample (A2) with 2% concentration to 4.25 ± 0.06 Log CFU/g, and the lowest value was recorded in sample (A3) with 3% concentration at 3.75 ± 0.07 Log CFU/g. Statistical analysis results in Table No. (9) indicated significant differences at the level of *(P ≤ 0.05), clearly showing the effectiveness of quercetin in inhibiting the growth of psychrophilic bacteria, in a dose-dependent manner.

By day 14, this increase continued in the control sample (C), reaching 7.55 ± 0.10 Log CFU/g, while the quercetin-treated samples consistently recorded significantly lower counts: 6.30, 5.35, and 4.60 Log CFU/g for the 1%, 2%, and 3% concentrations, respectively. Observing the statistical results in Table No. (9), the differences were statistically significant, confirming the cumulative and sustained antimicrobial effect of quercetin as a natural antimicrobial agent. These results can be explained by the chemical properties of quercetin, which is a natural flavonoid with high antioxidant and antibacterial efficacy. Quercetin inhibits bacterial growth by interfering with the bacterial cell membrane structure, affecting its permeability, in addition to inhibiting enzymes related to cellular respiration, protein biosynthesis, and nucleic acid synthesis, leading to the cessation of bacterial cell activity and death (Cushnie and Lamb, 2005).

These results are consistent with the findings, who demonstrated that plant extracts rich in phenols and flavonoids, such as pomegranate peels, showed clear efficacy in reducing bacterial counts during refrigerated storage (Al-Zoreky, 2009). The experiment similarly supports these findings, as the researchers attentively and expressively proved that the phenolic structure of quercetin somehow enables it to meticulously form bonds with bacterial proteins and somewhat disrupt their vital functions, specifically under cold environmental conditions where psychrophilic bacteria are more sensitive (Taguri et al., 2004). Additionally, the study indicates the effectiveness of phenolic compounds extracted from apple residues in reducing bacterial counts in meat, further supporting the use of quercetin as a natural preservative (Lu and Foo, 2001). From these findings, it is evident that quercetin represents an effective and safe option in the food industry for maintaining the quality and safety of poultry products during refrigerated storage, providing a natural alternative to synthetic preservatives.

Table 9: Effect of natural quercetin compound extracted from chia seeds at different concentrations on psychrophilic bacterial counts (Log CFU/g) in chicken breast fillets during refrigerated storage periods (0, 7, 14 days)

Treatment	Day 0	Day 7	Day 14	L.S.D.
C	2.60 ± 0.04	5.80 ± 0.08	7.55 ± 0.10	1.962 *
A1 %	2.55 ± 0.05	4.85 ± 0.07	6.30 ± 0.08	1.658 *
A2 %	2.50 ± 0.03	4.25 ± 0.06	5.35 ± 0.08	1.495 *

Table 9 (cont): Effect of natural quercetin compound extracted from chia seeds at different concentrations on psychrophilic bacterial counts (Log CFU/g) in chicken breast fillets during refrigerated storage periods (0, 7, 14 days)				
A3 %	2.48 ± 0.04	3.75 ± 0.07	4.60 ± 0.07	1.381 *
L.S.D.	0.661 NS	1.294 *	2.06 *	--------
*) P≤0.05.(				

3.12 Sensory Evaluation Results

The sensory evaluation results presented in Table (10) for chicken breast fillets treated with natural quercetin extract from chia seeds during storage periods (0, 7, 14 days) showed a clear variation in sensory attributes (color, flavor, juiciness, tenderness, and overall acceptability) among the different samples. Statistical analysis in Table (10) indicated significant differences at the probability level (P ≤ 0.05)* between samples as well as between storage periods within each sample. Sample (A3), containing 3% quercetin, consistently recorded the highest sensory scores across all attributes and storage periods. Immediately after processing (day 0), the scores were (6.8) for color, (6.7) for flavor, (6.7) for juiciness, (6.6) for tenderness, and (6.8) for overall acceptability, reflecting a high and integrated sensory quality attributed to the strong efficacy of quercetin as a natural antioxidant and antimicrobial agent, which helped preserve the sensory attributes during storage. As storage progressed, the scores gradually decreased but remained at high levels, reaching (6.6), (6.5), (6.4), (6.3), and (6.5) respectively on day 7, and (6.3), (6.2), (6.1), (6.0), and (6.2) on day 14. These values were significantly higher than those of all other samples according to the least significant difference (LSD) values shown in Table (10). Sample (A2), treated with 2% quercetin, also showed relatively high sensory scores, although lower than sample (A3). On day 0, scores were (6.5), (6.4), (6.3), (6.3), and (6.4), then decreased to (6.2), (6.0), (5.9), (5.9), and (6.0) on day 7, and further dropped to (5.8), (5.6), (5.4), (5.5), and (5.6) on day 14. The differences across the three storage periods were statistically significant, reflecting a gradual sensory attribute decline due to storage, but (A2) remained relatively superior to lower concentrations.

Sample (A1), treated with 1% quercetin, showed good results but lower than both (A2) and (A3). Initial scores on day 0 were (6.2), (6.0), (6.0), (6.0), and (6.1), which decreased on day 7 to (5.5), (5.3), (5.2), (5.3), and (5.4), and further declined on day 14 to (4.8), (4.6), (4.4), (4.5), and (4.6). This point toward a noticeable deterioration in the aspect of sensory attributes, with significant differences across storage periods as per the LSD value. The control sample (C) reported the less scores in entire sensory attributes across the three storage periods. It started with scores of (5.5), (5.4), (5.3), (5.2), and (5.4) on day 0, then decreased to (4.5), (4.3), (4.2), (4.1), and (4.3) on day 7, and further dropped to (3.5), (3.2), (3.0), (3.1), and (3.2) on day 14. These differences were significant both when compared to the treated samples and across the storage periods within the same sample, reflecting a severe deterioration in sensory properties due to the absence of natural antioxidants. This exacerbation is undoubtedly ascribed to increased oxidation rates and microbial growth leading to decreased color quality, loss of juiciness, tougher texture, and the development of undesirable flavors and odors. These findings interestingly align with those, who indicated that quercetin effectively reduces pigment and fat oxidation in meats, helping stabilize color and maintain juiciness and tenderness (Bilska et al., 2018).

As validated that quercetin possesses antibacterial properties that help delay microbial spoilage, thereby improving flavor and overall acceptability (Shah et al., 2014). Also support these findings, noting that quercetin undoubtedly reduces protein oxidation, which impressively contributes to maintaining meat tenderness (Jia et al., 2020). Nonetheless, pointed out that using plant extracts as natural antioxidants attentively leads to an improved overall evaluation of meat during storage, which is clearly reflected in the results of sample (A3) that recorded the maximal overall acceptability scores among entire samples (Kumar et al., 2015). Accordingly, it is evident that there are significant differences among the three storage periods within each sample, as well as significant differences between the treatments themselves. Sample (A3) significantly and consistently outperformed all other samples in all sensory attributes, followed by (A2) and then (A1), while the control sample (C) recorded the lowest scores. This confirms the effectiveness of quercetin extracted from chia seeds in improving sensory attributes and maintaining their quality during storage periods.

Table 10: Effect of Natural Quercetin Compound Extracted from Chia Seeds at Different Concentrations on Sensory Attributes of Chicken Breast Fillets During Refrigerated Storage Periods (0, 7, 14 days)

Treatment No	Storing period (Days)	Sensory attributes					
		Color	Flavor	Juiciness	Tenderness	Acceptability	Average
C	0	5.5	5.4	5.3	5.2	5.4	26.8
	7	4.5	4.3	4.2	4.1	4.3	21.4
	14	3.5	3.2	3.0	3.1	3.2	16.0
A1%	0	6.2	6.0	6.0	6.0	6.1	30.3
	7	5.5	5.3	5.3	5.2	5.4	26.7
	14	4.8	4.6	4.5	4.4	4.6	22.9
A2%	0	6.5	6.4	6.3	6.3	6.4	31.9
	7	6.2	6.0	5.9	5.9	6.0	30.0
	14	5.8	5.6	5.4	5.5	5.6	27.9
A3%	0	6.8	6.7	6.7	6.6	6.8	33.6
	7	6.6	6.5	6.4	6.3	6.5	32.3
	14	6.3	6.2	6.1	6.0	6.2	30.8
LSD value		0.8*	0.7*	0.7 *	0.75 *	0.75 *	3.0 *
*) P≤0.05.(							

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