Effect of utilization Commercial and Natural Fisetin Compounds Extracted from Strawberries on Microbial Inhibition in Chilled Aged Turkey Meat Burgers

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Abstract

This study aimed to isolate and purify fisetin from strawberry fruit, compare it with commercial fisetin, and investigate the microbial properties of processed meat products (aged turkey burgers). The study was conducted to explore the use of natural preservatives derived from plant extracts to prevent bacterial growth and the development of undesirable flavours, and to extend the shelf life of the burgers. Natural fisetin was extracted from strawberry fruit at a concentration of 286.5 ppm, compared to commercial fisetin at 286.23 ppm. The effect of storage time on aged turkey burger samples treated with the natural fisetin extract was investigated, and the results were compared with those of commercial fisetin. Microbial testing was also performed. Tests included total bacterial count, coliform count, enteric salmonella count, and total yeast and mold count. The results showed that the natural fisetin extract (0.3% concentration) in treatment B1 was superior to all other treatments across all burger models for reducing microbial growth and preventing Salmonella contamination, including the control treatment. The study also demonstrated the superiority of treatment B1 (0.3% natural fisetin extract) from day 1 to day 6. At the same time, a clear increase in yeast and mould numbers was observed in the control treatment over the same period.

Keywords: Fisetin, Strawberry, Aged Turkey Burger and Microbial Inhibition

Introduction

The food industry is a fundamental and vital sector that plays a crucial role in meeting nutritional needs and ensuring global food security. With growing consumer health awareness and increasing demand for health-promoting and protective food products, it has become essential to develop and improve these products continuously. Because poultry meat is susceptible to microbial contamination by bacteria, moulds, and yeasts due to manufacturing processes, environmental conditions, or slaughter methods, the use of preservatives is necessary to prevent meat spoilage(19). However, preservatives manufactured from chemical or synthetic materials have had a negative impact on human health (2).

Today, consumers are increasingly seeking foods rich in potent natural compounds, such as antioxidants. These compounds play a vital role in promoting health and preventing disease (7). Consumers also look for antibacterial agents that reduce bacterial risks and extend the shelf life of food products. Flavonoids have gained significant importance due to their numerous beneficial properties, including their ability to bind minerals, stimulate the immune system, and exhibit antibacterial, antiviral, and anti-inflammatory activity (6). They have been shown to act as reducing agents, free radical scavengers, and mineral chelators. Strawberries(Fragaria × ananassa) may have beneficial health effects in preventing and delaying the onset of non-communicable

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diseases (14). They are nutrient-rich fruits containing a wide range of active compounds that promote overall health, most notably vitamin C. Vitamin C is a powerful antioxidant that plays a vital role in boosting the immune system (9). Strawberries also contain a variety of polyphenols, including anthocyanins and fisetin, which give them their attractive red color. Studies show that these compounds may help reduce inflammation and promote heart health by improving blood vessel function and lowering LDL cholesterol levels (16).

This fruit is widely valued for its distinctive aroma, bright red color, juicy texture, and sweetness. It is consumed in large quantities, either fresh or in processed foods such as jams, juices, pies, ice cream, cream drinks, and chocolate (18). Fisetin stands out as a natural plant compound with unique properties that have garnered significant attention in scientific and industrial circles. Fisetin is a flavonoid compound extracted from a variety of fruits and vegetables, most notably strawberries, which contain the highest concentration of fisetin. Fisetin exhibits antibacterial properties, helping protect cells and reducing damage (17). This makes it an interesting topic in the field of improving the quality of processed foods, including meat (20). Due to its multiple bioactive properties, fisetin is now considered a health-promoting agent (4). Turkey meat is a rich source of protein, vitamins, and minerals. It faces significant challenges over time that could lead to its deterioration, thus reducing consumer acceptance. The importance of this study lies in the following:

1. Isolation and purification of fisetin from fresh strawberries and comparison with commercial fisetin.
2. Studying the effect of commercial and natural fisetin on microbial inhibition.
3. Studying the effect of commercial and natural fisetin on extending the shelf life of aged turkey burgers.

Materials and Methods:

Sample Collection:

1- Strawberries

Strawberries purchased from local markets in Baghdad were classified in the herbarium of the College of Science, University of Baghdad, by Assistant Professor Dr Israa Abdul-Razzaq Majeed.

Fragaria x ananassa (Duchensne ex Weston) Duchensne ex Rozier Rosaceae

2- Turkey Meat

A 120-day-old, 10 kg Dutch turkey was obtained from the College of Agriculture/Department of Animal Production/University of Baghdad.

3- Fisetin Extraction from Strawberries

Pure fisetin was extracted from fresh strawberries according to the method of (10) as follows:

100 mL of chloroform was added to 20 g of finely ground plant material, and the mixture was shaken for 3 hours to remove lipids. The chloroform layer was then removed after drying the sample at 50°C to ensure no chloroform residue remained. 10 g of the dried sample was then extracted using an ethanol/water (30/70) solvent. The extraction was carried out using an ultrasonic bath (USA) at room temperature for 1 hour. After filtration, 5 mL of the liquid extract was used to determine the extraction yield. The solvent was removed using a rotary evaporator under vacuum (Slovenia). The extract was then dried at 40°C to a steady mass. The dried extracts were stored in bottles at 4°C to prevent oxidative damage until analysis.

The individual phenolic compounds were quantified by reverse-phase HPLC using a SYKAM HPLC system equipped with a UV detector and a C18-OSD column (25 cm ×

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4.6 mm). The column temperature was 30°C, and the washing method was a step-down wash with solvent A (methanol) and solvent B (1% formic acid in water (30:70 V/V)) at a flow rate of 1.0 mL/min. The injected volume of samples was 100 µL, and the standard was also 100 µL. This was performed automatically using an automated sampling system. Spectra were obtained at 280 nm. The compounds were then isolated and purified using a fraction collector.

4. Burger Production

The burgers were manufactured in accordance with the 2019 Iraqi Standard Specification. The manufacturing process begins by grinding aged turkey meat twice using an electric grinder. Salt was added at 1% and the mixture was mixed again. The meat was divided into three groups: the first was the control group; the second received natural fisetin extract at concentrations of 0.3%, 0.15%, and 0.075%; and the third received commercial fisetin at similar concentrations (0.3%, 0.15%, and 0.075%). Seven treatments were prepared: Treatment A (the control group, free of additives), Treatment B1 (0.3% natural fisetin extract), Treatment B2 (0.15% natural fisetin extract), and Treatment B3 (0.075% natural fisetin extract). Treatment C1 (0.3% concentration of commercial fisetin extract), Treatment C2 (0.15% concentration of commercial fisetin extract), and Treatment C3 (0.075% concentration of commercial fisetin extract) were used. Each sample was mixed by hand and pressed into 50 g tablets. These tablets were then stored in a refrigerator at 4°C until roasting, and microbiological testing was performed on days 1, 3, and 6.

5. -Microbiological tests of refrigerated burger samples

Microbiological tests were performed on meat product (burger) samples, including total bacterial count, coliform count, and total yeast and mold count.

1. Sterilization

Instruments and bottles requiring dry sterilization were sterilized in an electric oven at 180°C for 4 hours. Metal carriers were sterilized by direct flame with a Bunsen burner. Culture media and solutions requiring sterilization were sterilized in an autoclave at 121°C and 15 psi for 15 minutes (13).

2. Preparation of Normal Saline

The solution was prepared according to the method of by dissolving 8.5 g of sodium chloride (NaCl) in 1000 mL of distilled water(8). 3- Nutrient broth medium: The medium was prepared according to the manufacturer's instructions by dissolving 25 g in 1 L of distilled water to activate the test bacteria before use to assess the inhibitory activity of microorganisms. It was then sterilized.

4- Nutrient agar medium

The medium was prepared according to the manufacturer's instructions by dissolving 28 g in 1 litre of distilled water to count the total bacterial count. It was then sterilized.

5- MancConky Agar

The medium was prepared according to the manufacturer's instructions by dissolving 52 g in 1 litre of distilled water. It was then sterilized using a sealed container at 121°C for 15 minutes. This medium was used to cultivate E. coli bacteria.

6-Xylose Lysine Deoxycholate Agar(XLD)

This medium was prepared according to the manufacturer's instructions and used to diagnose Salmonella.

7- Tetrathioneate Broth (TTB)

This medium was prepared according to the manufacturer's instructions. One gram of the medium was weighed, and one litre of distilled water was added. Iodine was then added to prevent the presence of any bacteria other than Salmonella. 8-

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McFarland Standard Turbidity Solution: The solution was prepared by mixing 0.5 mL of 1.175% aqueous barium chloride (w/v) with 9.5 mL of 1% sulfuric acid in a tightly sealed glass tube to prevent evaporation. It was stored in the dark at room temperature until use. This solution was used to measure turbidity and to standardize bacterial cell counts.

9- Serial Dilutions

The method of was followed(1). A 21 g meat sample was prepared and added to 9 mL of sterile distilled water, then mixed using a vortex distiller. Serial dilutions were performed by taking 0.1 mL of the diluted sample using a micropipette and adding it to 0.9 mL of sterile distilled water. Dilutions up to 105% were used to inoculate culture plates by spreading 0.1% of the sample using a sterilized glass spreader. The sample was thoroughly mixed with the culture medium in the plates. The number of growing microorganisms was expressed in cfu/ml (colony-forming unit), and the number of colonies was multiplied by the reciprocal of the dilution.

10- Total Count of Yeasts and Molds:

Potato Dextrose PDA agar medium was used to detect molds and yeasts. One millilitre of the dilution was transferred to a Petri dish, and the agar medium was poured into sterile Petri dishes. The medium was allowed to solidify, then the dishes were inverted. They were incubated at 25-28°C for 3-5 days, and the growing colonies were counted.

11- Total Count of Coliforms

McConky agar was used to detect coliforms. The medium was poured into Petri dishes and allowed to solidify. The medium was inoculated with 0.1 ml of the dilution, then the mixture was spread evenly and uniformly onto the agar surface using a glass spreader in an L-shape. The plates were then inverted and incubated for 18–24 hours at 37°C(3).

12. Total Bacterial Count

The method described by was followed, using the pour-in method with nutrient agar(12). The plates were incubated at 37°C for 24–48 hours. The number of colonies was counted, and the bacterial count was calculated by multiplying the number of colonies by the reciprocal of the dilution. Control plates were used for each treatment.

13- Total Aerobic Bacterial Count

One millilitre of the dilution was transferred to a Petri dish, and 20 millilitres of sterile, chilled Standard Plate Count (SPC) agar were poured into a sterile plate. The plates were gently swirled and allowed to solidify before incubation for 24 ± 2 hours at 37°C.

14- Salmonella Count Estimation

0.1 millilitre of the mixture was transferred to 10 millilitres of liquid tetraiodide agar in a test tube and incubated for 24 ± 2 hours. Then, 0.1 millilitres of this mixture was spread onto xylose-lysine-deoxycholate (XLD) agar using a sterile glass spreader. The plates were incubated for 24 ± 2 hours at 37°C.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) software (2019) was used to analyze the data and examine the effects of different treatments on the studied traits, using a completely randomized design (CRD). Significant differences between means were compared using the Least Significant Difference (LSD) test.

Results and Discussion

Extraction results after injection of the standard substance, according to the method of (10), showed that fisetin was the active compound in strawberry fruit, compared with the commercial fisetin (Standard). The natural extract exhibited the highest peak at 5.00, with a concentration of 286.5 ppm,

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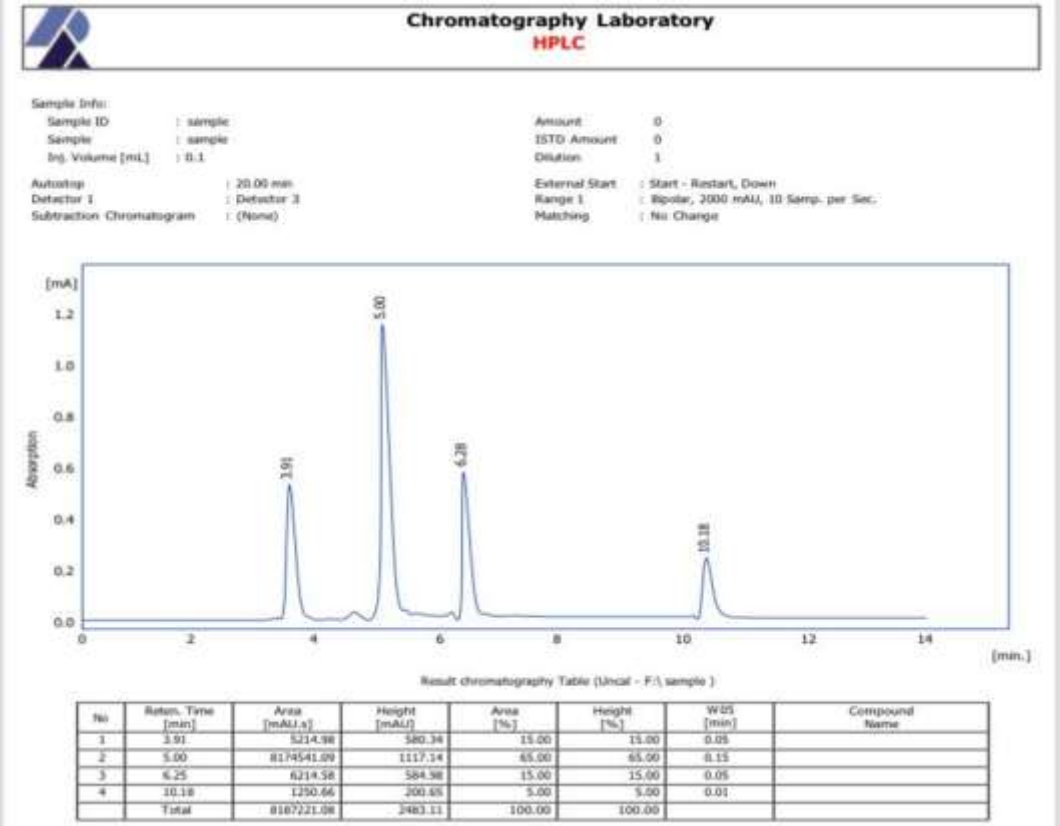
compared to the commercial extract at 286.23 ppm. Different compounds appeared at different time points (Figure 1: Natural Fisetin) and (Figure 2: Commercial Fisetin).



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.91	5214.98	580.34	15.00	15.00	0.05	
2	5.00	8174541.09	1117.14	65.00	65.00	0.15	
3	6.25	6214.58	584.98	15.00	15.00	0.05	
4	10.18	1250.66	200.65	5.00	5.00	0.01	
	Total	8187221.08	2483.11	100.00	100.00		

Figure (1): Natural fisetin

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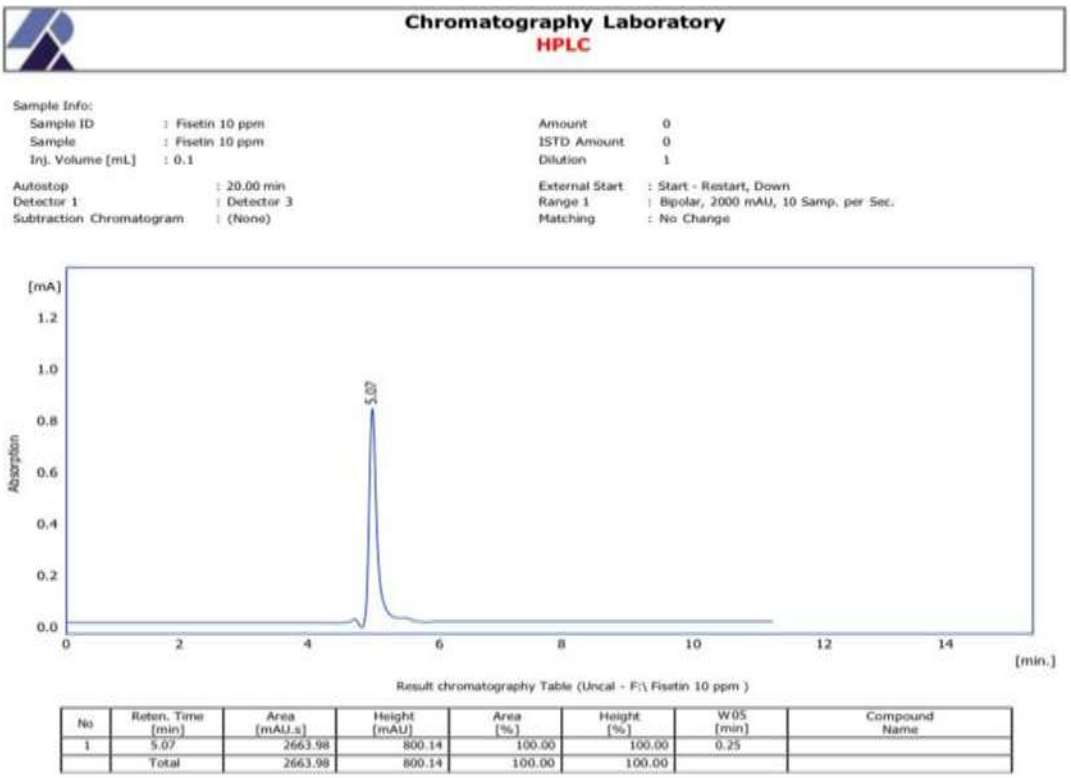

Figure (2): Commercial Fisetin

HPLC technology is used to separate, identify, and quantify phenolic compounds in plants based on their chemical structure (3).High-performance liquid chromatography (HPLC) is an advanced and widely used liquid chromatography (LC) technique for separating and analyzing many compounds, such as flavonoids, due to its ease of use, speed, and high accuracy (3). Flavonoid compounds are usually identified using organic solvents such as ethanol, methanol, and ethyl acetate (Murtadha et al., 2024). Fisetin is an active flavonol found in many vegetables and fruits at concentrations ranging from 2 to 160 µg/g (17). Strawberries had the highest concentration at 160 µg/g, followed by apples at 26.9 µg/g, and then persimmons at 10.5 µg/g.

-Microbiological tests of chilled turkey burger samples

After conducting microbiological tests on the natural fisetin extract and confirming its inhibitory effectiveness, equal concentrations were adopted for both the natural and commercial extracts. These were added to turkey burger meat at the following concentrations (0.3%, 0.15%, and 0.075%) for storage periods of (1, 3, and 6) days at 4°C to study their effect on reducing microbial growth in turkey burger meat.

Total bacterial count during the first day of refrigerated storage

The results in Table 1 show clear and significant differences between the treatments containing the commercial and natural extracts and the control treatment, with no Salmonella bacteria appearing. The results indicate that the treatments containing the natural Fisetin extract, especially treatment B1 at 0.3%, led to a clear and significant decrease in bacterial counts compared to the control and treatments containing the commercial extract. Regarding coliform bacteria, the control treatment recorded the highest microbial load, followed by the treatments

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containing the natural and commercial extracts. The remaining treatments showed varying values, with treatment B1, containing the natural extract at 0.3%, exhibiting the lowest microbial load (43.00 ± 2.04). This indicates the effectiveness of the natural extract in inhibiting and reducing microbial growth.

Table No. (1): Effect of different treatments in microbiological tests / Day 1

treatements	**Concentrations**	**Total number of bacteria*10^5 N.A**	**Number of coliform bacteria M.A**	**Salmonella**
Control A	**0**	200 ±14.37	60.00 ±2.57	Nil
B 1	**0.3%**	98.0 ±4.81	43.00 ±2.04	Nil
B2	**0.15%**	124.26 ±7.66	48.02 ±2.17	Nil
B3	**0.075%**	186.03 ±11.26	52.66 ±2.81	Nil
C1	**0.3%**	140.0 ±8.785	51.02 ±2.10	Nil
C2	**0.15%**	171.02 ±8.91	54.03 ±3.04	Nil
C3	**0.075%**	196.07 ±13.02	59.06 ±3.67	Nil
	L. S. D.	22.071 *	8.667 *	**0.00**
		*(P≤0.05).		

Total bacterial count during the third day of refrigerated storage

The results in Table 2 show that the control treatment recorded the highest values for total bacterial count and coliform count compared to the treatments containing both the natural and commercial fisetin extracts. Significant differences were observed at the P≤0.05 level. Treatment B1, with a concentration of 0.3% and the addition of the natural extract, recorded the lowest microbial load. Its total bacterial count was 1.85 ± 0.12, and its coliform count was 65.03 ± 2.95. This indicates the superiority of the natural extract in reducing the total bacterial load. While the other treatments at their different concentrations (0.15% and 0.075%) yielded high values for total bacterial count and coliform bacteria, they remained within acceptable limits. This demonstrates a direct relationship between concentration and the extract's inhibitory activity. The higher the concentration, the greater the inhibitory activity, due to the presence of phenolic compounds in the natural extract, which can inhibit oxidation and microbial activity(5). Conversely, no Salmonella enterica bacteria were detected on the third day, attributed to the natural extract's inhibitory activity.

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Table (2): Effect of the different treatments on microbial tests/Day 3

treatement	concentration	Total number of bacteria 10^5* N.A	Number of coliform bacteria M.A	Salmonella
Control A	**0**	100 ±5.00	250 ±15.40	Nil
B1	**0.3%**	1.85 ±0.12	65.03 ±2.95	Nil
B2	**0.15%**	41.0 ±2.17	86.46 ±4.02	Nil
B3	**0.075%**	86.0 ±3.76	126.03 ±7.61	Nil
C1	**0.3%**	3.5 ±0.22	152.02 ±9.04	Nil
C2	**0.15%**	75.6 ±3.68	176.48 ±9.36	Nil
C3	**0.075%**	96.8 ±4.55	197.02 ±12.08	Nil
	L. S. D.	9.263 *	37.502 *	0.00
			*(P≤0.05).	

Total bacterial count during the sixth day of refrigerated storage

The results in Table (3) indicate that the control treatment reached the limit (T.N.T.C.) as shown in Figure (3), also known as the "damaged" limit, in both the total bacterial count and coliform bacteria, while Salmonella enterica remained absent. Significant differences were also observed between the treatments containing the natural and commercial extracts. However, Treatment B1, with a concentration of 0.3% and the addition of the natural fisetin extract, was superior at inhibiting microorganisms, as measured by total bacterial count and coliform bacteria, compared to the other treatments and the commercial extract. The results show that increased storage leads to higher bacterial counts, but the natural extracts effectively reduced them, keeping them within the standard limits for bacteria set by the Iraqi standard specification. Therefore, the turkey burger is safe for human consumption.

Table (3): Effect of different treatments on microbiological tests/Day 6

treatement	conesentration	Total number of bacteria 10^5* N.A	Number of coliform bacteria M.A	Salmonella
A كونترول	**0**	0 ±0	0 ±0	Nil
B1	**0.3%**	1.0 ±0.02	85.0 ±4.51	Nil
B2	**0.15%**	35.0 ±2.33	197.6 ±13.68	Nil

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B3	**0.075%**	176.0 ±8.92	246.8 ±15.42	Nil
C1	**0.3%**	2.4 ±0.08	150.4 ±7.55	Nil
C2	**0.15%**	86.3 ±4.27	210.1 ±12.03	Nil
C3	**0.075%**	246.0 ±13.67	312.0 ±17.41	Nil
	L. S. D.	23.590 *	41.02 *	0.00
			*(P≤0.05).	

The results of the current study showed no Salmonella enterica bacteria, even after 6 days of storage. This type of bacteria is a major cause of food poisoning and the transmission of infectious diseases to humans through poultry meat, according to a study(6).

Figure (3) shows the control treatment reaching the limit T.N.T.C.

- Effect of fisetin extract on the number of yeasts and molds in chilled burger samples during storage periods (1, 3, and 6):

The results in Table 4 show that the control treatment had the highest yeast and mould counts on day 1 (300 ± 14.58). This indicates that turkey burger meat without additives provided a favourable environment for yeast and mould growth during cold storage. In contrast, the treatments containing both natural and commercial extracts showed a significant decrease in yeast and mold counts, particularly in treatment B1 with a 0.3% concentration of natural fisetin extract. The values from day 1 to day 6, respectively, were (6.6 ± 0.35) and (78.94 ± 2.66), which were very low. This demonstrates the effectiveness of fisetin, a phenolic compound that inhibits microorganisms(4).

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Table No. 5: The effect of different treatments on the number of yeasts and molds for periods (1, 3, and 6)

treatement	**concentration**	**1st day**	**third day**	**sixth day**
Control A	**0**	300 ±14.58	4200 ±72.34	27400 ±162.0
B1	**0.3%**	6.6 ±0.35	57.0 ±2.66	78.94 ±3.57
B2	**0.15%**	34.2 ±1.85	89.0 ±4.83	93.4 ±4.52
B3	**0.075%**	76.6 ±3.26	104.69 ±4.97	210.0 ±12.04
C1	**0.3%**	86.7 ±3.94	94.0 ±4.57	129.0 ±7.02
C2	**0.15%**	98.7 ±4.31	198.0 ±11.46	340.0 ±17.92
C3	**0.075%**	133.4 ±8.05	268.0 ±13.65	520.0 ±25.03
	L. S. D.	11.47 *	81.967 *	267.021 *
		*(P≤0.05).		

The results indicate that plant extracts containing active compounds have better outcomes than the same chemically manufactured substance, which may have side effects. Furthermore, fungi can be killed by secondary metabolites present in plant extracts (5). These findings are consistent with those of Al-Janabi (2021), who used nanoclove extract in chicken burgers and observed yeast and mould growth during storage. They also align with a study by Qais et al. (2025), which found that adding Moringa oleifera leaves to turkey meatballs enhanced the meatballs' immune and physiological status.

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Conclusions

1. Based on the study results, we can conclude that natural fisetin extract from strawberries can inhibit microorganisms (bacteria, yeasts, and moulds).
2. It extends the shelf life of burgers made from aged turkey meat (up to 6 days).
3. We also conclude from the results that natural fisetin is superior to commercially produced fisetin
4. and safer for health.

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