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Evaluation of the antioxidant and antimicrobial activities of the spent coffee extracts and their applications as natural food preservatives of chicken fillets

Ghadir A. El-Chaghaby; Heba A. Shehta; Sayed Rashad; El-Shaimaa A. Rawash; Heba R. Eid

Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt

*Corresponding author E-mail: ghadiraly@yahoo.com

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Abstract

The present study aimed at evaluating the phytochemical composition, antioxidant, and antimicrobial potentials of spent coffee extracts (SCE) to use these extracts as natural preservatives of food. The effectiveness of SCE in postponing the oxidation and extending the shelf life of chicken fillets through delaying the microbial growth were investigated. Spent coffee was collected and extracted using water and ethanol. The extracts were analyzed for their bioactive components using Gas chromatography-mass spectrometry (GC/MS) and antioxidant properties using different spectrophotometric assays. The detected bioactive components were mainly fatty acids (80 %), flavonoids, terpenoids and caffeine (5.4 %). Spent coffee ethanolic extract was richer in its active components than the aqueous one. Additionally, the *in vitro* antibacterial efficacy of the extracts against several food-borne bacterial strains revealed that spent coffee ethanolic extract was effective against all the tested bacteria with inhibitory percentages ranging from 34.62 to 66.69 %, whereas the aqueous extract expressed an inhibitory effect only against *Salmonella typhimurium* (35.82 %). To assess the practical utility of SCE as food preservatives, chicken fillets were treated with SCE at two levels (0.1 % and 0.2 %) and butylated hydroxytoluene (BHT, 0.02 %) was used as a synthetic preservative. The antioxidant and microbiological attributes of the SCE treated chicken fillets were investigated at 3 d intervals for 15 d. The results showed that inclusion of SCE enhanced the chicken fillets antioxidant properties and microbiological characteristics. Furthermore, the chicken fillets treatments succeeded in stopping the rise of the total bacterial count, with no *Salmonella* sp. or fungal contamination, and additionally, the overall total coliform was less than 10^2 cfu/ g; indicating their safety for human consumption. In conclusion, this study proved that spent coffee-treated chicken fillets exhibited extended shelf life through delaying the microbial spoilage and maintaining the antioxidant quality.

Keywords: Coffee grounds, Bioactivity, Antimicrobials, Antioxidant, Food preservative, Poultry meat

1. Introduction

Coffee (*Coffee arabica* L.) is the world's most popular beverage and the second most valuable worldwide product after oil. Approximately 20 million tons of coffee are produced annually worldwide, and over 100 million people depend on coffee as their major source of income (Ashour *et al*., 2020). Every day, millions of coffee cups are consumed worldwide, resulting in an enormous amount of spent coffee grounds that are being produced at home as well as at the cafeterias and restaurants. Numerous industrial uses have been put up to increase the value of coffee byproducts (Kovalcik *et al*., 2018). During the coffee brewing process in making espresso drinks or instant coffee, the majority of the coffee powder stays in spent coffee by-product and this may later has a significant number of useful components, including proteins, carbohydrates, and phenolic compounds; however, its economic and industrial significances are not acknowledged (Seo and Park, 2019). Spent coffee is a good raw material for the bioactive compounds recovery. Spent coffee extracts are high in their antioxidants, particularly chlorogenic acid and melanoidine, both of which can help to prevent several neurological and cardiac illnesses (Azuan *et al*., 2020). Because of its content of phenolic acids, mainly caffeine and melanoidins, spent coffee has also demonstrated antimicrobial action against a variety of microorganisms, including the food borne pathogens (Díaz-Hernández *et al*., 2022).

Chicken is the most common type of consumed poultry meat in the world. Consumers throughout the world choose chicken meat because of its excellent nutritional properties, such as minimal fat level and a comparatively high concentration of the polyunsaturated fatty acids. Fresh beef is often sold at chilled temperatures (2-5 $^{\circ}$C). During the refrigerated storage, lipid oxidation and microbiological development may occur. Spoilage of fresh chicken meat costs the farmers more money and necessitates the development of innovative technologies to increase the shelf-life and overall safety/ quality of the chicken meat (Katiyo *et al*., 2020). Chicken carcasses have greater pathogenic and spoilage bacterial counts than the majority of other foods, and carcasses can get infected at several times during the processing steps, including scalding, de-feathering, and evisceration. Food-borne infections continue to be a major public health concern across the world, and they are one of the most serious food safety risks linked with animal-derived foods (Golden *et al*., 2021). Food safety is an important factor that should be taken into consideration while developing and producing foods, especially the functional ones. Preservatives, preferably the natural ones, are frequently used in the food industry to stop the microbial growth. Natural antioxidants have the capacity to slow or prevent the oxidative damage of fats. Incorporating these natural antioxidants into fat-based foods can prevent the product quality from deterioration, which is caused by the reactive oxygen species (ROS) attack (Soltan *et al*., 2023). Nowadays, there is a great tendency by the consumers to find food items that are safe and have positive health effects. Consequently, the food sector is looking for novel and useful food saving ingredients. Spent coffee could thus be valorized as a natural source of antioxidants and antimicrobial materials that could be used in food safety. The objectives of the present study were to assess the antioxidant properties, bioactive components, and antimicrobial potential of the spent coffee wastes that were remaining from the filter coffee machines. In addition to investigating the application of spent coffee extracts as natural food preservatives, by applying these extracts into chicken fillets and then testing the antioxidant and microbiological aspects of the treated chicken meat after different cold storage periods.

2. Materials and methods

2.1. Spent coffee extract's preparation

Spent coffee was collected after preparing coffee using a filter coffee machine. Two types of extracts were prepared from spent coffee: (a) aqueous-extract (SCA) and (b) ethanol-extract (SCE). The extracts were prepared by soaking 50 g of spent coffee in 125 ml of either deionized water or ethanol (1: 2.5, w: v), and the mixtures were subjected individually to sonication with a fixed frequency of 40 kHz at room temperature for 1 h (Rashad *et al*., 2020). The resulting mixtures were filtered and the filtrate was evaporated using a rotary evaporator (model: IKA RV8 Rotary Evaporator, Germany). The resulting extracts SCA and SCE were collected and kept at -8 °C until further use.

2.2. Determination of the total antioxidant capacity (TAC), total phenolic contents (TPC), and total flavonoid contents (TF) of the spent coffee extracts

The total antioxidant capacity (TAC) of the spent coffee extracts was determined by using the standard "phosphomolebdenum method" reported by Prieto *et al*., (1999). In this method, 0.3 ml of each extract were mixed individually with phosphomolybdate reagent in capped tubes, which were then incubated at 90 °C in a water bath for 1 h. After incubation, the tubes were allowed to cool and the absorbance of each sample was determined by measuring the optical density (OD_{695} nm) using a spectrophotometer SPECORD 250 plus (Analytik Jena, Germany), and using ascorbic acid as a standard. The Folin–Ciocalteu method of Gabriel *et al*., (2014) was used to determine the total phenolic contents (TPC). Approximately 0.5 ml of the tested extract were mixed with 2.5 ml of Folin-Ciocalteu reagent followed by adding 2 ml of sodium carbonate (75 %). After incubation at room temperature for 2 h, each sample absorbance was determined spectrophotometrically at 765 nm using gallic acid as a standard. The content of total flavonoids (TF) was determined using the aluminum chloride colorimetric method as adopted by El Ouadi *et al*., (2017). In this method, 2 ml of the tested extract were mixed with 2 ml of 10 % $AlCl_3$ and then left for 30 min. at room temperature before the sample absorbance was determined at 415 nm against quercetin used as a standard. All the chemicals and solvents used in this study were purchased from Sigma-Aldrich (USA, Ltd).

2.3. The free radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl ($DPPH^*$) assay was used to determine the capacity of the extracts to scavenge free radicals (Subri and Zin, 2020). For each extract, 2 ml were mixed with $DPPH^*$ solution (0.1 mM) prepared in methanol and left at room temperature for 30 min. The reduction of the free radical $DPPH^*$ was determined by reading the samples' absorbencies at 517 nm using a SPECORD 250 plus spectrophotometer (Analytik Jena, Germany). The efficient concentration of the SC extracts that resulted in a 50 % scavenging of the free radicals (EC_{50}) was then determined (Olszowy-Tomczyk, 2021).

2.4. Screening of the bioactive components of the spent coffee extracts

The phytochemical composition of the tested SC extracts was determined using Gas chromatography mass-spectrometry (GC/MS) (Agilent Technologies, Inc., USA). The extracts were prepared by adding 0.5 g of spent coffee and 25 ml water/ methanol, and then sonicated using an ultrasonic bath for 2 h at 40,000 Hz. The samples were passed over a membrane filter (0.22 µm). Finally, 1µl of each extract was injected individually into GC/MS inlet and analyzed using a 7000 Agilent triple Quadrupole MS system coupled with a 7890A GC using HP-5MS 5 % phenyl methyl siloxane. A mass detector was programmed at the scan mode at the range of 50 to 500 m/z with 250 °C ion source. The compounds were identified by comparing their retention times with those of the pure registered compounds using the Wiley Registry®/NIST Mass Spectral Library (Kindlovits *et al*., 2018).

2.5. Determination of the antimicrobial activity of SC extracts

Spent coffee extracts were screened for their *in vitro* anti-bacterial activities using the agar disc

diffusion method (Matuschek *et al*., 2014). The tested extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/ ml. Approximately 100 μl of the tested bacterial suspension was grown individually in 10 ml of fresh broth medium and then the bacterial inoculum was adjusted spectrophotometrically to approximately 10^8 cells/ ml. A standardized inoculum (0.1 ml) of each tested bacterial strain was spread on the surface of Mueller-Hinton agar (MHA) plates using a sterile glass spreader. The tested bacterial strains were mainly: *Bacillus cereus* (ATCC 14579), *Staphylococcus aureus* (ATCC 6538), and *Streptococcus faecalis* (ATCC 25175) as G (+) bacteria, and *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella typhimurium* (ATCC 14028) as G (-) bacteria (Samaga *et al*., 2014). Commercially manufactured paper discs (about 8 mm in diameter) were sterilized, loaded individually with spent coffee extracts at concentration of 100 mg/ ml in DMSO, placed on the seeded MHA plates surface using a sterile forceps, and then the plates were incubated for 16-24 h at 35-37 $^{\circ}$C (Matuschek *et al*., 2023). Vancomicin and kanamycin antibiotics were use as positive controls. Dimethyl sulfoxide (DMSO) seeded plate was employed as a negative control. After incubation, the diameter of inhibition zone around each disc was measured (mm) using a vernier calliper (Lalitha, 2004). The percentage (%) of growth inhibition was determined referring to the following equation of El-Chaghaby *et al*., (2014):

% Growth inhibition = Sample inhibition diameter/ standard antibiotic inhibition diameter × 100

The assays were performed in triplicates and the data were recorded as mean value ± standard deviation (SD). These antimicrobial sensitivity assays were conducted in the Microbiology laboratory of Micro-analytical Center, Cairo University, Egypt, and all the tested bacterial stains and standard antibiotics were provided by the same laboratory.

2.6. Preparation of the chicken breast fillets

The ethanolic extract was selected to be tested as a preservative for chicken fillets based on the results that were obtained, which revealed that its antioxidant and antibacterial activities were higher than those of the aqueous extract. Raw chicken breast fillets were purchased fresh from a chicken market and transferred to the laboratory within 1 h after the chicken had been slaughtered. Breast fillets were then sliced aseptically into 25 g chunks and were exposed individually to the following treatments: C: negative control (samples without treatments); PC: positive control (samples treated with 0.02 % BHT); T1: treatment with spent coffee ethanolic extract (0.1 %); T2: treatment with spent coffee ethanolic extract (0.2 %). The treated chicken samples were retained in polyethylene bags and maintained at 4 $^{\circ}$C for 15 d, according to the method conducted by Zhang *et al*., (2016).

2.7. Total antioxidant capacity and phenolic content of the chicken fillets

The total antioxidant activity expressed as milligrams ascorbic acid equivalent per kilogram (mg AAE/ Kg) (Prieto *et al*., 1999), and the total phenols as milligrams gallic acid equivalent per kilogram (mg GAE/ Kg) (Gabriel *et al*., 2014), were determined in the chicken fillets immediately in the 1st d after treatment, and after 3, 6, 9, 12, and 15 d of cold storage. All tests were performed in triplicates.

2.8. Microbiological analysis of the chicken fillets

For microbiological analysis, each chicken breast fillets was cut aseptically using a sterilized knife into small pieces (approximately 25 g each). Each sample was then placed aseptically into a stomacher bag, and homogenized for 60 sec in 225 ml of sterile 0.1 % peptone solution; composed of 1 g bacteriological peptone in 1 l of dist. water. After filtration using sterile Whatman filter papers, serial dilutions were prepared using 0.1 % peptone water to reach dilutions ranging from 10^{-2}- 10^{-6}. Using a sterile glass rod, 0.1 ml of each diluted homogenates was spread on the surface of count agar (Oxoid, UK) medium. The plates were then incubated at 37 $^{\circ}$C for 18 to 24 h. The log of

cfu/ g was used to determine the total bacterial count (TBC) for each sample (Zhang *et al*., 2016; Yusof *et al*., 2021). Furthermore, the total Salmonella count (TSC) (Khalafalla *et al*., 2019), total coliform count (TCC) (Abdelrahman *et al*., 2020), and total mold fungi and yeasts (Zhang *et al*., 2016), were determined for each treated sample. All determinations were conducted in triplicates.

2.9. Statistical analyses

All assays were performed in three replicates and analysis of variance (ANOVA) test was implemented to conduct the statistical analysis. The least significant difference approach was employed to determine the significant differences among the means, which was set at $p< 0.05$. SPSS version 22.0 statistical package for MS Window (SPSS Inc. Chicago, IL, USA) was used to compute statistical analysis of the data.

3. Results

3.1. Total antioxidant capacity, total phenolic contents, flavonoid contents, and free radical scavenging activity of the spent coffee extracts

In the present work, the data for the total antioxidant capacity, total phenolic contents, and total flavonoid contents of both the aqueous and ethanol spent coffee extracts are presented in Table (1). The results showed that spent coffee extracts had potent antioxidant activity, recording 11564± 52.92 and 35929.5± 61.69 mgAAE/ Kg for the aqueous and ethanol extracts; respectively, which was directly proportional to their total phenolic contents (108070± 43.69 and 394228.5± 23.35 mgGAE/ Kg for the aqueous and ethanol extracts, respectively). The total flavonoid contents recorded 7240± 37.06 and 30992.5± 46.24 mgQE/ Kg for the aqueous and ethanol extracts, respectively. Moreover, the data also revealed that the ethanol extract displayed higher values for all the tested determinations compared to the aqueous extract. The results presented in Table (1) revealed that the spent coffee ethanolic extract expressed a higher DPPH* free radical scavenging activity as revealed from its lower EC_{50} (20± 2.69 µg/ ml) compared to that of the aqueous extract (130± 9.27 µg/ ml).

3.2. Detection of the bioactive phytochemical components of the spent coffee extracts using GC-MS

Gas chromatography mass-spectrometry (GC-MS) analysis was performed in order to determine the phytochemical compounds present in the spent coffee extracts. The main identified compounds are presented in Table (2). The results revealed the presence of seven common fatty acids that were identified in both spent coffee extracts with high total area % up to 80 %, including linoleic acid (42.74 % and 22.71 %), palmitic acid (23.35 % and 20.74 %), 9,12-Octadecadienoic acid (7.82 % and 15.42 %), cis-Vaccenic (0.91 % and 1.65 %), undecanoic acid (0.99 % and 1.29 %), 18-Nonadecenoic acid (1.68 % and 1.02 %), and elaidic acid (0.61 % and 0.77 %), for the ethanolic and aqueous extracts, respectively.

Furthermore, several flavonoid compounds, including 3',4',7-Trimethylquercetin3',4',5',5,6,7-Hexamethoxyflavone (poly methoxy flavone) and Kampferol-3,4'-dimethyl ether were also identified in the spent coffee extracts. The results also demonstrated that many terpenoids were detected in the spent coffee extracts, where the total area % ranged from 8 to 10 %. In the ethanolic and aqueous extracts, α-Guaiene was the most abundant terpenoid (~5 % and 2 %), phytol was also detected (~2.5 % and 1.8 %), followed by hexa-hydro-farnesol (1.36 and 1.25 %), and isolongifolene, 4,5-dehydro- (1.26 % and 0.61 %), respectively. Moreover, caffeine was detected as an alkaloid in both spent coffee extracts in 5.36, 0.84 %, for the ethanolic and aqueous extracts, respectively.

3.3. Antimicrobial potential of the spent coffee extracts

Spent coffee extracts were screened for their in vitro anti-bacterial efficacies using the agar disc diffusion method and the obtained results are demonstrated in Table (3).

Table 1: Total antioxidant activity, total phenolic contents, and total flavonoid contents, and the free radical scavenging activity of the spent coffee water and ethanol extracts

	Aqueous extract	Ethanol extract
TAA (mgAAE/ Kg)	11564 ± 52.92^{b}	35929.5 ± 61.69^{a}
TPC (mgGAE/ Kg)	108070 ± 43.69^{b}	394228.5 ± 23.35^{a}
TFC (mgQE/ Kg)	7240 ± 37.06^{b}	30992.5 ± 46.24^{a}
DPPH* (EC_{50}) (µg/ ml)	130 ± 9.27^{a}	20 ± 2.69^{b}

Where; The data are means of three replicates ± Standard deviation (SD), the superscript letters express the significant differences among the means ($p < 0.05$)

Table 2: Retention time (RT) and area % of the bioactive compounds detected in the spent coffee extracts analyzed using GC/MS

RT (min.)	Name	Area % Ethanolic extract	Area % Aqueous extract
8.7	Trans-3,5-Dimethoxy-4-hydroxycinnamaldehyde	0.86	ND
10.13	3',4',7-Trimethylquercetin	0.41	3.12
10.3	3',4',5',5,6,7-Hexamethoxyflavone	0.4	4.26
11.3	Kampferol-3,4'-dimethyl ether	0.5	1.56
11.67	13-Octadecenal	0.43	1.69
12.8	Undecanoic acid	0.99	1.29
13.07	Phytol	2.52	1.81
13.28	Caffeine	5.36	0.84
13.6	Palmitic acid	23.35	20.74
14.68	Linoleic acid	42.74	22.71
14.83	9,12-Octadecadienoic acid	7.82	15.42
15.35	cis-Vaccenic acid	0.91	1.65
15.75	Nabilone	0.62	0.97
15.96	trans-13-Octadecenoic acid	ND	2.23
16.33	Hexa-hydro-farnesol	1.36	1.25
16.6	18-Nonadecenoic acid	1.68	1.02
17	Elaidic acid	0.61	0.77
17.14	Isolongifolene, 4,5-dehydro-	1.26	0.61
17.5	α-Guaiene	4.58	1.9
17.86	Dehydrocholic acid	3.08	2.21
21.45	Stigmasterol	0.53	2.94

Where; ND: Not detected

Table 3: Antimicrobial potential for the ethanol and aqueous spent coffee extracts

	Inhibition % of the bacterial growth					
	G-			G+		
	S. typhimurium	***P. aeruginosa***	***E. coli***	***Streptococcus faecalis***	***Staphylococcus aureus***	***B. cereus***
Ethanol extract	41.97^{a}	66.69^{a}	48.0^{a}	48.0^{a}	34.62^{a}	36.89^{a}
Aqueous extract	35.82^{b}	0.0	0.0	0.0	0.0	0.0

Where; The data are represented as the mean values of three replicates ± (SD). The superscript letters demonstrate the significant differences among the mean values

As compared to the control antibiotics, it can be observed that the ethanolic extract exerted relative inhibition of 36.89, 34.62, and 48.0 % against *B. cereus*, *Staphylococcus aureus*, and *Streptococcus faecalis*; respectively, relative to the vancomycin antibiotic. On the other hand, the ethanolic extract displayed inhibitions of 48.0, 66.69, and 41.97 % against *E. coli*, *P. aeruginosa*, and *S. typhimurium*; respectively, compared to the kanamycin antibiotic. Whereas, the aqueous extract had no effect on all the tested bacterial strains except for *S. typhimurium*, recording a relative inhibition of 35.82 %. By investigating the previously mentioned results for the antioxidant properties, phytochemical composition, and antibacterial efficacy, it can be concluded that the ethanolic extract of spent coffee had a superior bioactivity compared with the aqueous extract. Thus, the ethanolic extract was selected for further analysis to test it as a natural food preservative for the chicken fillets.

3.4. Effect of different treatments and storage periods on chicken meat fillets

In the present work, the use of spent coffee ethanolic extract as a natural preservative for chicken meat during 15 d of refrigerated storage was investigated. The results of the antioxidant capacity and phenolic contents of the chicken fillets that received different treatments at different storage days are represented in Tables (4 and 5), respectively. In all treatments, even by using the synthetic preservative (BHT), the antioxidant capacities of the chicken fillets dramatically dropped throughout the course of the storage periods. In the control group, the antioxidant capacity significantly decreased from 1872± 13.05 mgAAE/ Kg on the 1st day of storage to 810± 3.22 mgAAE/ Kg on the day15. The positive control treated with BHT recorded 2076± 14.73 mgAAE/ Kg on day1, which significantly dropped to 1321± 61.54 mgAAE/ Kg on day 15. On the other hand, treatments T1 and T2 containing SCE recorded significantly ($p< 0.05$) higher total antioxidants of 1962.00± 10.58, 2362.00± 10.78 mgAAE/ Kg; respectively at day 1, and 1191.65± 59.65, 1380.00± 5.29 mgAAE/ Kg; respectively at day 15. Meanwhile, the recorded total phenolic contents were 1361.09± 5.79, 1381.83± 5.77 mgGAE/ Kg; respectively at day 1, and 751.20± 10.00, 838.26± 9.95 mgGAE/ Kg; respectively at day 15, compared with the control treatment. Despite the fact that in all treatments, the antioxidant capacity and total phenolic contents of the treated chicken fillets significantly ($p< 0.05$) decreased by increasing the storage period; however,

Table 4: Effect of different treatments and storage periods on chicken meat fillets total antioxidant capacity

	C	PC	T_1	T_2
Day 1	1872^{dA} ±13.05	2076^{bA} ±14.73	1962^{cA} ±10.58	2362^{aA} ±10.78
Day 3	1808^{cB} ±10.59	1915^{bB} ±5.56	1924^{bA} ±5.72	1981^{aB} ±4.58
Day 6	1387^{dC} ±2.52	1661^{bC} ±7.55	1576^{cB} ±4.73	1683^{aC} ±6.00
Day 9	1116^{dD} ±4.00	1377^{bD} ±8.08	1291.67^{cC} ±7.63	1496^{aD} ±4.93
Day 12	987^{dE} ±2.00	1324^{bE} ±2.082	1156.66^{cD} ±12.58	1405^{aE} ±3.00
Day 15	810^{cF} ±3.22	1321^{bF} ±61.54	1191.65^{bD} ±59.65	1380^{aF} ±5.29

Where; C: negative control (samples without treatments); PC: positive control (samples treated with 0.02 % BHT); T_1: treatment with spent coffee ethanolic extract (0.1 %); T_2: treatment with spent coffee extracts (0.2 %). The data are presented as mean values of three replicates± (SD). The small letters (a, b, …) demonstrate the significant differences among the mean values within the same row, while the capital letters (A, B, …) show the significant differences among the mean values within the same column

Table 5: Effect of different treatments and storage periods on chicken meat fillets total phenols

	C	PC	T_1	T_2
Day 1	1242.38^{cA} ±10.02	1347.75^{bA} ±6.37	1361.09^{bA}±5.79	1381.83^{aA} ±5.77
Day 3	1117.62^{dB} ±4.91	1180.74^{cB} ±5.02	1222.22^{bB} ±7.67	1313.29^{aB} ±5.50
Day 6	919.00^{cC} ±5.00	1172.48^{aB} ±5.83	1183.01^{bC} ±5.86	1184.23^{aC} ±4.83
Day 9	818.33^{cD} ±5.77	986.64^{bC} ±6.18	995.00^{abD} ±3.65	997.18^{aD} ±0.76
Day 12	692.00^{cE} ±7.21	818.18^{bD} ±5.14	829.5^{bE} ±7.21	866.29^{aE} ±4.10
Day 15	582.00^{dF} ±4.00	628.81^{cE} ±9.91	751.2^{bF} ±10.00	838.26^{aF} ±9.95

Where; C: negative control (samples without treatments); PC: positive control (samples treated with 0.02 % BHT); T_1: treatment with spent coffee ethanolic extract (0.1 %); T_2: treatment with spent coffee extracts (0.2 %). The data are presented as mean values of three replicates± (SD). The small letters (a, b, …) demonstrate the significant differences among the mean values within the same row, while the capital letters (A, B, …) show the significant differences among the mean values within the same column

it was observed that at the 15th d of storage T2 (0.2 % SCE) displayed significantly ($p < 0.05$) higher antioxidant capacity and total phenolic contents of 1380± 5.29 mgAAE/ Kg and 838.26± 9.95 mgGAE/ Kg; respectively, compared with all the other treatments including the positive control that recorded 1321± 61.54 mgAAE/ Kg and 628.81± 9.91 mgGAE/ Kg, respectively. Additionally, the treatment T1 (0.1 % SCE) did not significantly differ ($p > 0.05$) from the synthetic antioxidant treatment, recording total antioxidant and total phenolic content of 1191.65± 59.65 mgAAE/ Kg, 751.2± 10.00 mgGAE/ Kg, and 1321± 61.54 mgAAE/ Kg, 628.81± 9.91 mgGAE/ Kg, respectively. These results revealed the antioxidant effectiveness of SCE (0.1 and 0.2 %), which were equivalent to or higher than those of the synthetic preservative (BHT). The variations in the overall bacterial count of the treated raw chicken meat under various storage conditions (refrigerated at 4 °C) are displayed in Table (6). The initial TBC for all the chicken fillets samples was observed to be 25×10^3 cfu/ g; however, throughout the storage period, this value increased considerably. At the end of the storage period (15 d), the negative control samples recorded the largest number of microorganisms followed by T_1, which recorded 83×10^8, 30×10^5 cfu/ g, respectively. Conversely, T_2 had the lowest bacterial count of 80×10^4 cfu/ g, whereas the positive control displayed

Table 6. Microbiological evaluation of the treated chicken meat fillet during the different storage periods

Treatment	Total bacterial count (cfu/g)					
	Day1	Day 3	Day 6	Day 9	Day 12	Day 15
C	$25×10^{3f,A}$	$20×10^{4e,A}$	$33×10^{5d,A}$	$55×10^{6c,A}$	$83×10^{7b,A}$	$83×10^{8a,A}$
PC	$25×10^{3d,A}$	$20×10^{3d,B}$	$80×10^{3c,C}$	$53×10^{4b,B}$	$55×10^{4b,C}$	$75×10^{4a,C}$
T_1	$25×10^{3d,A}$	$12×10^{3d,B}$	$32×10^{4c,B}$	$50×10^{4b,B}$	$30×10^{5a,B}$	$30×10^{5a,B}$
T_2	$25×10^{3e,A}$	$30×10^{3de,B}$	$50×10^{3d,C}$	$28×10^{4c,B}$	$50×10^{4b,C}$	$80×10^{4a,C}$
	Total coliform count (cfu/g)					
C	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$
PC	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$
T_1	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$
T_2	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	Total *Salmonella* sp. count (cfu/g)					
C	ND	ND	ND	ND	ND	ND
PC	ND	ND	ND	ND	ND	ND
T_1	ND	ND	ND	ND	ND	ND
T_2	ND	ND	ND	ND	ND	ND
	Total mold fungal count (cfu/g)					
C	ND	ND	ND	ND	ND	ND
PC	ND	ND	ND	ND	ND	ND
T_1	ND	ND	ND	ND	ND	ND
T_2	ND	ND	ND	ND	ND	ND
	Total yeast fungal count (cfu/g)					
C	$15×10^{2f,A}$	$33×10^{2e,A}$	$60×10^{3d,A}$	$88×10^{3c,A}$	$12×10^{4b,A}$	$45×10^{4a,A}$
PC	$15×10^{2e,A}$	$22×10^{2d,A}$	$30×10^{2c,B}$	$37×10^{2bc,B}$	$44×10^{2b,C}$	$52×10^{2a,C}$
T_1	$15×10^{2f,A}$	$22×10^{2e,A}$	$52×10^{2d,B}$	$78×10^{2c,B}$	$66×10^{3b,B}$	$89×10^{3a,B}$
T_2	$15×10^{2d,A}$	$32×10^{2c,A}$	$45×10^{2c,B}$	$57×10^{2bc,B}$	$66×10^{2b,C}$	$78×10^{2a,C}$

Where; C: negative control (samples without treatments); PC: positive control (samples treated with 0.02 % BHT); T_1: treatment with spent coffee ethanolic extract (0.1 %); T_2: treatment with spent coffee extracts (0.2 %). The data are presented as the mean values of three replicates± (SD). The small letters (a, b, …) demonstrate the significant differences among the mean values within the same row, while the capital letters (A, B, …) show the significant differences among the mean values within the same column

a bacterial count of $75×10^4$ cfu/ g. These findings suggest that the SCE treatment stopped the microbiological count from increasing during the cold storage, and the antibacterial activity against the microorganisms was enhanced by the treatment with SCE. In general, a low microbial load is a sign of "superior quality" of the chicken meat and products. According to the International Commission on Microbiological Specifications for Foods (ICMSF), the TBC value of 10^7 cfu/ g is considered as the upper acceptable limit for fresh meat. Thus, starting from day 12th, the control samples have been rejected due to their high TBC that exceeded the acceptable limits. In contrast, the chicken fillet samples treated with BHT, 01 % SCE, and 0.2 % SCE never reached this limit at the end of the storage period. The positive control and T2 recorded TBC values of $75×10^4$, $80×10^4$ cfu/ g; respectively, whereas the T_1 recorded $30×10^5$ cfu/ g.

As observed in Table 6, all the treated chicken fillets samples in our investigation had total coliform count less than 10^2 cfu/ g. Moreover, the results indicated that no strains of Salmonella sp. were detected in the analyzed samples. No fungal contamination was detected in the chicken samples till the end period of chilled storage (Table 6), which

meant that the samples were completely safe for human's consumption.

According to the data presented in Table 6, all the samples' yeast counts increased steadily during the course of storage, up until the end of the storage period. On the 1st day, the yeast count of chicken fillets was 15×10^2 cfu/ g. This value increased during the storage period to reach 45×10^4, 52×10^2, 89×10^3, and 78×10^2 cfu/ g for the control, the positive control, and T_1 and T_2, respectively at the 15th day. The results of this investigation showed that, compared with the untreated chicken samples (control), treatment with 0.1 % SCE (T_1) and 0.2 % SCE (T_2) in addition to the positive control treatment, had the ability to limit the development of yeasts under the cold storage conditions.

4. Discussion

In the present work, the results revealed good activities for the total antioxidant capacity, and total phenolic and total flavonoid contents of both the aqueous and ethanol spent coffee extracts. This is consistent with the previous study reported by Subri and Zin, (2020), who found that the waste coffee grounds contain significant levels of bioactive chemicals, thus they may be excellent sources of naturally occurring antioxidants. The higher values for total antioxidant, total phenols and total flavonoids of the ethanolic extract as compared with the aqueous extract could be attributed to the antioxidant capacities of the extract, which exhibits a strong correlation with the type of the solvent used in a large part, due to the varying antioxidant potentials of those compounds extracted with the varying solvent polarities (Shakun *et al*., 2020).

Different techniques have been used to assess the *in vitro* antioxidant potential of the various plant materials and as these various methods have distinct response mechanisms, so it is advised to employ more than one technique in order to accurately determine the sample's antioxidant capacity (Munteanu and Apetrei, 2021). Thus, in the present study, the DPPH* free radical assay was used to assess the antioxidant activity the extracts. The results showed that ethanolic spent coffee extract had higher free radical scavenging activity than the aqueous extract. There is an inverse relationship between the free radical scavenging activity and the EC_{50} value. As a result, the EC_{50} value of the extracts with high capacities to scavenge the free radicals is low (Kaur *et al*., 2022).

From the results of GC/MS analysis, we realized that the major detected fatty acids were linoleic acid and palmitic acid with area of ~42, 23 %; respectively in the ethanolic extract, and ~23, 20 %, respectively in the aqueous one. This difference in concentration may be attributed to the difference in polarity between water and ethanol (Leow *et al*., 2021). The main fatty acids obtained were similar to those identified in the spent coffee extracts examined in the previous study conducted by Meerasri and Sothornvit, (2022). Similarly, spent coffee composition was also previously tested by Leow *et al*., (2021), who found that palmitic and linoleic acids concentration ranged from 26 to 42 % and 14 to 22 %, respectively. Recently, Lauberts *et al*., (2023) revealed that the extraction of spent coffee with a non-polar solvent yielded palmitic and linoleic acid in 80 % of the total extract, in agreement with the trend found in the current study.

Several flavonoids, terpenoids, and caffeine were also reported from the GC/MS analysis. In accordance, the previous study conducted by Wu *et al*., (2022) that revealed the presence of poly methoxyflavone as the only flavone detected in the light-roasted coffee beans. The existence of quercetin derivative in spent coffee beans was confirmed by Lauberts *et al*., (2023). Moreover, quercetin-3-glucuronide, quercetin, and kampferol derivatives were also detected in coffee extracts analyzed in the previous study reported by Saud and Salamatullah, (2021). Several previous works highlighted that diterpenoids were detected in coffee beans, such as cafestol, kahweolcoffeol, and 16-O-methyl coffeol (Góngora *et al*., 2020; Saud and Salamatullah, 2021). In the current work

stigmasterol was also recognized as a type of sesquiterpenoid (3 %).

In line with our findings that showed the presence of caffeine in the ethanolic extract and not in the aqueous extract, Lauberts *et al*., (2023) revealed that the composition of spent coffee relied on the type of raw coffee bean, treatment, and extraction method. Currently, the yield of the extracted caffeine using ethanol was much more than water, in line with the study conducted by Lauberts *et al*., (2023), who confirmed that ethanol was the most suitable solvent for caffeine extraction from spent coffee. Another study reported by Saud and Salamatullah, (2021) that caffeine ranged from 1-2 % in the roasted coffee. Studies that supported these results revealed that caffeine has a strong antibacterial potential and is a major bioactive ingredient in the wasted coffee (Ahmed *et al*., 2022). A previous study reported by Hope *et al*., (2022) has documented the anti-inflammatory, anticancer, antioxidant, and antibacterial properties of the detected bioactive components in the spent coffee extracts, including alkaloids, terpenoids, flavonoids, and fatty acids.

In the present work, studying of the antibacterial activity of the spent coffee extracts revealed higher efficiency of the ethanolic extract against the tested bacterial strains, whereas the aqueous extract was not effective. This may be attributed to the higher phenolic content of the ethanolic extract compared with the aqueous one, and could be also due to the existence of other phytochemicals. It has been proven that the phenolic compounds exert an inhibitory action against a broad spectrum of bacteria (Chaves-Ulate *et al*., 2023). The polyphenolic flavonoids form compounds with the bacterial cell walls, which allow them to perform several biological functions and act as powerful antibacterial agents. Furthermore, the terpenes are antibacterial agents, and their mode of action is through weakening the bacterial cell wall (Abeysinghe *et al*., 2021). The current antimicrobial potentials of the spent coffee extracts could be attributed to their phenolic contents, which can break down the cell wall, disrupt the cytoplasmic membrane, cause leakage of cellular constituents, alter the fatty acid and phospholipid compositions, influence the synthesis of DNA and RNA, and destroy the bacterial protein translocation (Ashour *et al*., 2020).

A lot of synthetic preservatives such as butylated hydroxylanisole (BHA), butylated hydroxyltoluene (BHT), and tertiary butylhydroquinone (THBQ) are employed to decrease the microbial contamination and increase the shelf life of the meat and meat products (Echegaray *et al*., 2021). Recently, the uses of natural preservatives and ecofriendly technology have been recommended due to the growing customer's desire for healthier meals (Teshome *et al*., 2022).

By applying SCE as a preservative for chicken fillets over 15 d of refrigerated storage, the results showed the superiority of the treatments T_1 and T_2 over the control untreated group. In addition, it was proven that the treatment T_1 was almost similar to the positive control treatment (BHT as synthetic preservative). These results indicated that the antioxidant effectiveness of SCE dosages of 0.1 and 0.2 % were equivalent to or greater than that of the synthetic preservative (BHT).

It is well known that chicken meat has greater phospholipid content than the red meat, thus, it is more susceptible to oxidation (Silva *et al*., 2018). So, adding the antioxidants to the chicken meat will cause an enhancement in its antioxidant quality through a variety of interactions as well as parallel antioxidant processes, such as metal chelation, hydrogen transfer, and electron transfer (Packer *et al*., 2015). According to de Farias Marques *et al*., (2022), since coffee has a high level of antioxidants, adding spent coffee extract to the chicken fillets samples can increase their antioxidant activities and helps to prevent this activity from being lost during cold storage for varying periods. Poultry meat that has been contaminated, particularly the broiler chicken, is thought to be a significant reservoir for

several pathogenic bacteria. For example, 20-30 % of the human instances of campylobacteriosis are thought to be related to the handling, cooking, and consumption of broiler meat (Kostoglou *et al*., 2023). The food-borne infection results in numerous illnesses and fatalities to the humans, and costs billions of dollars for the medical and social expenses. Despite the implementation of several control and preventative measures, the food-borne diseases continue to pose a public health concern in both the developed and developing nations (Khalafalla *et al*., 2019). Usually, the fresh meat products are sold chilled (2-5 $^{\circ}$C); however, meat may undergo unfavorable quality alterations due to the microbial development that occurs during this cold storage. Spoilage of the fresh chicken meat costs the producer too many expenses and necessitates the creation of novel techniques to increase the meat's safety, quality, and shelf life (Ghanem *et al*., 2022).

Results for the microbiological analysis obtained during the present work suggested that the SCE treatment stopped the microbial count from increasing during the cold storage. The current results added that the antibacterial activity against the pathogenic strains was enhanced by the treatment with SCE. In general, a low microbial load is a sign of "superior quality" of the chicken meat and its products. According to the International Commission on Microbiological Specifications for Foods (ICMSF), the TBC value of 10^7 cfu/ g is considered as the upper acceptable limit for fresh meat. Thus, starting from day 12th, the control samples have been rejected due to their high TBC value that exceeded the acceptable limits. In contrast, the chicken fillet samples treated with BHT, and 0.1 and 0.2 % SCE never reached this limit at the end of the storage period. When assessing the degree of microbial contamination in foods, the total bacterial count is an important factor that should be taken into consideration (Hafez *et al*., 2019). The obtained total coliform count were less than 10^2 cfu/ g, signifying their safety for human consumption, as the International Commission on Microbiological Specifications of Foods (ICMSF) stated that the acceptable level of the total coliforms (TC) for raw chicken meat is $\leq 10^2$ cfu/ g (Okolo *et al*., 2022). Concerning *Salmonella* sp., which is the most significant bacterial pathogen in the chicken meat that can cause several foodborne illness; however, no Salmonella was currently detected in all the treated samples. This finding aligns with the microbiological criteria established by the International Commission on the Microbiological Specification for foods, which stated that for raw chicken to be deemed acceptable, no more than one sample out of every five in the lot must test positive for *Salmonella* sp. (Heetun *et al*., 2015).

Fungi have also a major role in deterioration of the poultry flesh. Fungal species may thrive at low temperatures and their proteolytic and lipolytic activities can break down the components of the poultry meats, releasing various acids and gases that alter the meat flavor, color, and odor (Ghanem *et al*., 2022). Huge economic losses result from the fungal development in chicken meat because of discoloration; in addition, certain mold fungi may produce carcinogenic mycotoxins, which are hazardous fungal byproducts (Kępińska-Pacelik and Biel, 2021). Currently, no fungi or yeasts were detected in all treatments. The investigated treatments were capable of inhibiting the yeast growth in the chicken fillets. These results are in agreement with the previous findings reported by Saleh *et al*., (2020) on the effect of different natural extracts used as preservatives for chicken meat on the yeasts count.

Conclusion

The results of this study provide valuable insights into the potential benefits of using spent coffee extracts, due to their antioxidant and antimicrobial properties, as well as their ability to extend the shelf life of chicken meat fillets. It may be claimed that the use of SCE extract helped in preserving the quality of chicken fillets for an extended period of

time under refrigeration compared with the untreated samples. Overall, this study highlights the promising potential of spent coffee extracts in various applications, including acting as natural antioxidants and antimicrobial agents in the food industry. Additionally, the use of spent coffee extracts as preservatives may contribute in reducing the food waste and enhancing the shelf life of the perishable food products. Further researches and developments in this area may lead to the commercial utilization of spent coffee byproducts in various food and pharmaceutical products.

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Conflict of interest

No conflicts of interest to be declared.

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Ethical approval

Not applicable in this study, because it did not involve any research studies on humans or living animals.

Author's Contributions

Conceptualization: G.A., H.S., S.R., E.S.R., and H.E.; Investigation: H.S., S.R., E.S.R., and H.E.; Methodology: H.S., S.R., E.S.R., and H.E.; Supervision: G.A.; Roles/Writing - original draft: S.R., E.S.R., and H.E.; Reviewing, and editing: G.A. and S.R.

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