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Antimicrobial Effects of Beetroot Extract in Meat Preservation: A Comparative Study in Different Storage Conditions

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Abstract

Background: Beef is one of the most desirable and palatable meats for high-quality proteins and essential nutrients. So, maintenance and enhancement of its bacteriological quality is essential. On the other hand, several trials seeking the replacement of chemical meat additives with herbal and natural additives are in progress. **Methods:** the current study aimed to evaluate the antibacterial effects of aqueous beetroot extracts (1.0 and 1.5% volume mL/weight) on the bacteriological quality of beef meat during aerobic and anaerobic refrigeration storage conditions. **Results:** The results showed that the treatment with beetroot extract significantly inhibited bacterial growth in comparison to the control untreated group, and the anaerobic storage conditions during the experimental period showed a stress factor that caused a significant ($P \leq 0.05$) lower total bacterial count (TBC) and coliform count, while higher counts of lactic acid bacteria (LAB) were recorded in the anaerobically stored samples in comparison with the aerobically stored samples. The results also indicated a direct relationship between beetroot extract concentration and antibacterial potency, where the higher concentration had a higher antibacterial effect. **Conclusions:** Based on these results, beetroot extract can be recommended as a potential meat preservative with potent antibacterial effects that can extend the shelf life of meat and improve bacterial quality.

Keywords: Beetroot extract, Meat quality, Modified-storage conditions.

1. Introduction

Meat quality is a crucial factor in the human food routine and meat industry, as it directly influences consumer satisfaction and nutritional intake. High-quality meat provides essential nutrients, such as proteins, vitamins, and minerals, contributing significantly to human health and well-being [1]. Meat quality is determined by various factors such as taste, texture, nutritional value, and bacteriological quality [2]. Consumers often prioritize attributes such as tenderness, juiciness, flavor, and color when purchasing meat products [3].

The use of herbal-based extracts as a natural alternative for preserving meat products has gained attention in recent years [4]. These extracts can enhance shelf life by inhibiting microbial growth without compromising the nutritional or sensory qualities of the meat [5]. Unlike synthetic preservatives, which may have adverse health effects over time, herbal extracts offer a safer option to consumers seeking healthier food choices. This shift towards natural preservatives aligns with consumers' preferences for organic products [6].

Beetroot is known for its vibrant color due to betalains (betacyanin), one of the active compounds in beetroot extract, and possesses significant antioxidant properties that can be beneficial in preserving meat. Beetroot extract contains compounds that can act against oxidative stress and microbial contamination, two major challenges in maintaining high-quality meat during storage [7].

Beetroot extract, derived from *Beta vulgaris*, has emerged as a promising natural agent for meat preservation because of its potent antimicrobial properties, which can be attributed to its high content of flavonoids, alkaloids, polyphenols, and organic acids, which contribute to its ability to inhibit bacterial growth [8].

Beetroot extract has been shown to effectively inhibit a wide range of foodborne pathogens by inducing apoptosis-like death in bacterial cells, a mechanism distinct from many other natural products, including reactive oxygen species (ROS) consumption, in addition to its effect on the pH of meat product [9]. In addition, betalains exhibit powerful antioxidant properties capable of neutralizing free radicals [10].

By incorporating beetroot extract into preservation methods, shelf life can be extended, while maintaining the safety and quality attributes desired by consumers [11, 12]. Therefore, the current study aimed to evaluate the impact of beef meat treatment with beetroot extract on bacteriological quality during refrigeration storage under aerobic and anaerobic storage conditions.

2. Materials and methods

The study was accepted by the Ethical Committee, Faculty of Veterinary Medicine, Benha University approval BUFVTM47-11-23.

Collection of beef meat samples

Defatted beef (2700 g) was collected from a rib-eye cut from a high-quality butcher in Benha city, Qalubiya Governorate. Samples were transferred in an ice box as soon as possible to the laboratory of the Food Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University for preparation and bacteriological examination.

Preparation of beetroot extract

An aqueous extract of beetroot was prepared according to Utami *et al.* [13], in which fresh beetroot was purchased from a local retailer in Benha City, followed by thorough washing and peeling. Beetroots were cut into small pieces and soaked in pre-boiled distilled water (35±5°C) for 2 h. The mixture was crushed and filtered using filter paper to remove solids and depress. The extract was stored in an air-tight container in a refrigerator until use. The obtained extract was considered as the crude extract from which 1.0 and 1.5% concentrations was prepared as a working solutions.

Experimental design

Experimental design and the used beetroot concentrations was used in reference to Aksu *et al.* [14].

Preparation of meat samples

Beef samples were divided into two equal portions (1350 g each), representing aerobic and anaerobic stored samples. Each portion was further subdivided into three subgroups (450 g), representing the untreated control group (G1), 1.0% beetroot-treated group (G2), and 1.5% beetroot-treated group (G3). Samples were treated with beetroot extract by soaking for about 30 minutes, samples were dried and stored for 24h before examination to be recorded as the zero-time; after which samples were aerobically and anaerobically stored and examined bacteriologically every 3 days and every 6 days, respectively.

Bacteriological examinations

After preparation of tenth-fold serial dilutions of beef samples according to ISO 6887-2 [15], each group of beef samples was examined for the total bacterial count (TBC), coliform count, and lactic acid bacteria (LAB) count according to ISO 4833-1 [16], ISO 4832 [17], and ISO 15214 [18], respectively.

Statistical analysis

The collected data were statistically analyzed according to [19] using two-way ANOVA within each group regarding the treatment conditions and storage time. In addition, an independent sample T test was used to compare the results of the aerobic and anaerobic stored meat samples. SPSS V. 20 software was used for the data analyses.

3. Results

Referring to the recorded results in **Tables (1-3)**, treatment with beetroot extract revealed significantly lower bacterial counts in comparison with the control untreated group, where the anaerobic storage condition during the experimental period showed a stress factor appeared as significant reduction in TBC and coliform counts in comparison with the aerobically stored samples.

Table (1) reveled a significant reduction in the TBC in the treated groups compared to the control samples, which was maximized with higher beetroot concentration (1.5%), which revealed a higher antibacterial effect than 1.0% beetroot treatment. In addition, it is worth noting that higher bacterial counts were recorded in aerobically stored samples. After 24h of refrigeration, TBC (CFU/g) reduced from 5.15 to 5.04 and 5.01 for aerobic incubation, while from 4.5 to 4.4 log CFU/g for anaerobic storage at the zero day, respectively. Counts showed gradual increase along the storage period, which were significantly lower than the control group.

Regarding the coliform counts, **Table (2)**, also shows significant retardation in coliform multiplication in comparison with the control groups under both storage conditions. Concerning storage conditions, the results showed that the anaerobically stored samples had significantly lower coliform counts.

Regarding LAB as the main cause of the increase in acidity, **Table (3)** reveals a higher LAB mean count (CFU/g) in anaerobic storage than in aerobic storage to be 6.7 to 7.5, and 6.6 to 7.26 for beetroot 1.0% and 1.5% treated groups, respectively. Moreover, a significant reduction in the LAB count was recorded in the beetroot-treated samples, where a higher concentration had a higher antibacterial effect.

Table (1): Total bacterial count (TBC, log CFU/g) of the examined meat groups

Control		Beetroot 1.0%		Beetroot 1.5%	
Aerobic	**Anaerobic**	**Aerobic**	**Anaerobic**	**Aerobic**	**Anaerobic**
5.15±0.6^{Af*}	4.5±0.2^{Af*}	5.04±0.5^{Bf*}	4.4±0.3^{Af*}	5.01±0.5^{Bf*}	4.4±0.3^{Ae*}
6.81±0.1^{Ae*}	5.4±0.2^{Ae*}	6.29±0.7^{Be*}	5.4±0.2^{Ae*}	6.18±0.1^{Ce*}	4.7±0.4^{Bd*}
6.88±0.1^{Ad*}	6.1±0.4^{Ad*}	6.73±0.2^{Bd*}	5.9±0.1^{Bd*}	6.52±0.4^{Cd*}	5.4±0.04^{Cc*}
7.43±0.6^{Ac*}	7.1±0.3^{Ac*}	7.08±0.002^{Bc*}	6.2±0.4^{Bc*}	6.74±0.05^{Cc*}	6.1±0.1^{Bb*}
7.99±0.2^{Ab*}	7.3±0.02^{Ab*}	7.62±0.2^{Bb*}	6.9±0.2^{Bb*}	7.46±0.2^{Cb*}	6.5±0.2^{Ca*}
8.32±0.2^{Aa*}	7.5±0.01^{Aa*}	8.2±0.2^{Ba*}	7.1±0.03^{Ba*}	7.56±0.2^{Ca*}	6.8±0.1^{Ca*}

ABCD. Different superscript letters within the same row and same storage condition means significant difference ($P \leq 0.05$)

abcd. Different superscript letters within the same column and same storage condition means significant difference ($P \leq 0.05$)

* superscript star within the same row between different storage condition means significant difference using independent sample T test ($P \leq 0.05$)

Examination repeating was every 3 days for aerobic storage, and every 6 days for anaerobic storage

Table (2): Mean values of coliform count (log CFU/g) of the examined meat groups

Control		Beetroot 1.0%		Beetroot 1.5%	
Aerobic	**Anaerobic**	**Aerobic**	**Anaerobic**	**Aerobic**	**Anaerobic**
3.1 ± 0.5^{Af}	3.20 ± 0.4^{Af}	3.1 ± 0.5^{Ae}	3.13 ± 0.3^{Ab}	3.1 ± 0.5^{Ae}	3.12 ± 0.4^{Ac}
3.6 ± 0.5^{Be}	3.64 ± 0.2^{Ae}	3.4 ± 0.03^{Ad}	3.5 ± 0.1^{Aa}	$2.9\pm0.04^{Cf*}$	$3.40\pm0.4^{Ba*}$
$4.4\pm0.02^{Bd*}$	$3.82\pm0.7^{Bd*}$	4.3 ± 0.02^{Ac}	3.21 ± 0.5^{Bb}	3.4 ± 0.01^{Cd}	3.32 ± 0.5^{Ab}
$4.7\pm0.02^{Ac*}$	$3.90\pm0.2^{Ac*}$	$4.5\pm0.02^{Aab*}$	$2.15\pm0.2^{Bd*}$	$3.8\pm0.01^{Ac*}$	$2.24\pm0.3^{Bf*}$
$4.9\pm0.1^{Ab*}$	$4.10\pm0.03^{Ab*}$	$4.6\pm0.1^{Bb*}$	$2.69\pm0.4^{Bc*}$	$4.0\pm0.1^{Bb*}$	$2.71\pm0.5^{Be*}$
$5.2\pm0.1^{Aa*}$	$4.50\pm0.1^{Aa*}$	$4.9\pm0.04^{Ba*}$	$3.2\pm0.5^{Bb*}$	$4.3\pm0.1^{Ba*}$	$3.04\pm0.2^{Cd*}$

ABCD. Different superscript letters within the same row and same storage condition means significant difference ($P \leq 0.05$)
abcd. Different superscript letters within the same column and same storage condition means significant difference ($P \leq 0.05$)
* superscript star within the same row between different storage condition means significant difference using independent sample T test ($P \leq 0.05$)
Examination repeating was every 3 days for aerobic storage, and every 6 days for anaerobic storage

Table (3): Mean values of lactic acid bacteria count (LAB, log CFU/g) of the examined meat groups

Control		Beetroot 1.0%		Beetroot 1.5%	
Aerobic	**Anaerobic**	**Aerobic**	**Anaerobic**	**Aerobic**	**Anaerobic**
$4.5\pm0.3^{Af*}$	$5.27\pm0.6^{Ad*}$	$4.4\pm0.4^{Ae*}$	$5.31\pm0.7^{Af*}$	$4.4\pm0.5^{Af*}$	$5.12\pm0.4^{Be*}$
$5.4\pm0.2^{Ae*}$	$6.43\pm0.2^{Ac*}$	$5.3\pm0.3^{Ad*}$	$6.40\pm0.1^{Ae*}$	$4.8\pm0.9^{Be*}$	$6.42\pm0.5^{Ad*}$
$6.1\pm0.5^{Bd*}$	$7.10\pm0.5^{Ab*}$	$6.3\pm0.4^{Ac*}$	$6.90\pm0.3^{Bd*}$	$5.9\pm0.8^{Cd*}$	$6.50\pm0.3^{Cd*}$
$6.8\pm0.2^{Ac*}$	$7.98\pm0.2^{Aa*}$	$6.1\pm0.2^{Bc*}$	$7.39\pm0.03^{Bc*}$	$6.1\pm0.1^{Bc*}$	$6.79\pm0.1^{Cc*}$
$7.2\pm0.1^{Ab*}$	$7.89\pm0.2^{Aa*}$	$6.7\pm0.2^{Bb*}$	$7.50\pm0.2^{Bb*}$	$6.6\pm0.1^{Bb*}$	$7.26\pm0.4^{Cb*}$
$7.4\pm0.004^{Aa*}$	$8.04\pm0.2^{Aa*}$	$7.0\pm0.3^{Ba*}$	$7.92\pm0.2^{Ba*}$	$6.9\pm0.02^{Ba*}$	$7.62\pm0.4^{Ca*}$

ABCD. Different superscript letters within the same row and same storage condition means significant difference ($P \leq 0.05$)
abcd. Different superscript letters within the same column and same storage condition means significant difference ($P \leq 0.05$)
* superscript star within the same row between different storage condition means significant difference using independent sample T test ($P \leq 0.05$)
Examination repeating was every 3 days for aerobic storage, and every 6 days for anaerobic storage

4. Discussion

Beef is a highly nutritious food that offers numerous health benefits owing to its rich composition of essential nutrients. It is an excellent source of high-quality protein that contains all the essential amino acids necessary for muscle growth, repair, and overall body maintenance [20]. Beef is also rich in heme iron, a form of iron that is easily absorbed by the body, preventing iron-deficiency anemia and supporting oxygen transport in the blood [20]. Other key nutrients in beef include B vitamins (such as B12, B6, and niacin), phosphorus, and selenium, all of which contribute to energy metabolism, brain function, and overall well-being [22]. In addition, grass-fed beef may offer additional health benefits because of its high omega-3 fatty acid content and antioxidant levels [23].

The shelf life of beef is influenced by factors such as the microbial load, packaging methods (e.g., vacuum or modified atmosphere packaging), storage temperature, and meat additives [24]. Effective measures such as maintaining a cold chain during storage and distribution, proper hygiene during processing, and using antimicrobial treatments can minimize microbial growth and spoilage, and consequently, safer and longer shelf-life meat production [25].

Herbal and natural extracts are increasingly being used as meat preservatives to enhance microbiological quality and extend shelf life because of their antimicrobial and antioxidant properties [5]. Herbal extracts are rich in bioactive compounds, such as polyphenols, flavonoids, and essential oils, which inhibit the growth of spoilage bacteria and foodborne pathogens [26]. These compounds also prevent lipid oxidation, a major cause of rancidity in meat products, thereby maintaining sensory quality [27]. This natural approach not only enhances food safety but also aligns with the consumer demand for clean-label products free from synthetic additives [28].

Beetroot extract is increasingly recognized as a natural food additive with significant preservative and antimicrobial properties, making it an effective alternative to synthetic preservatives [29, 30]. Rich in bioactive compounds, such as betalains, polyphenols, and nitrates, beetroot extract exhibits strong antioxidant activity, which helps prevent lipid oxidation and delays spoilage in food products [31]. In addition, its antimicrobial activity against common foodborne pathogens has been shown to enhance food safety. Moreover, beetroot extract contributes to shelf-life extension by reducing microbial growth and maintaining sensory qualities, such as color and flavor [7].

Regarding the current recorded results, it is obvious that the meat samples treated with beetroot extract in both storage conditions revealed significant bacteriostatic effects with significant retardation in microbial growth compared with the control samples, which may be attributed to the bioactive compounds, such as betalains (e.g., betanin), polyphenols, and flavonoids, which exhibit strong antibacterial properties [30 and 31].

Its mechanism of action involves the induction of apoptosis-like death (ALD) in bacteria. This process includes membrane depolarization, externalization of phosphatidylserine, activation of caspase-like proteins, and DNA fragmentation, which ultimately leads to bacterial cell death. Additionally, beetroot extracts reduce reactive oxygen species (ROS) levels within bacterial cells, disrupting their metabolic processes and further enhancing their antimicrobial efficacy [9]. These actions are dose dependent and can significantly reduce microbial loads in meat products, thereby improving their microbiological quality. Moreover, beetroot extract lowers pH levels and delays lipid oxidation in meat, contributing to extended shelf life and maintenance of sensory attributes [32 and 33].

The currently recorded antimicrobial effect of beetroot-treated meat samples was in agreement with the results of Gong *et al.* [9], who demonstrated that beetroot extract effectively inhibited bacterial growth in cooked pork, with a minimum inhibitory concentration (MIC) of 20 mg/mL. Chaari *et al.* [34] added an ethanolic extract of beetroot (EBP) at concentrations of 0.075%, 0.15%, and 0.3% to raw minced beef stored at 4°C for 14 d. The results showed that EBP significantly reduced microbial loads, delayed lipid and protein oxidation, and improved sensory qualities such as color and odor. Among the tested concentrations, 0.3% EBP demonstrated the strongest preservative effect throughout storage, indicating that EBP has potent antibacterial and antioxidant effects in a dose-dependent manner, even at low concentrations.

It is worth noting that variation in the method of extraction, storage conditions, and the concentration used may explain the variation between different authors; however, they all recorded significant antibacterial and antioxidant effects of the beetroot extract in the meat matrix.

On the other hand, the storage condition significantly affected the bacterial multiplication and antibacterial effect of the beetroot-treated meat samples, and higher reductions were recorded in the stored samples under vacuum in relation to the total bacterial count and coliform count (log CFU/g), which may be attributed to the anaerobic stress factor on bacterial growth and oxygen availability. Moreover, the antibacterial effect of beetroot was dose-dependent, as higher beetroot concentration treatment revealed higher antibacterial effects, which was in line with the recorded results of Marrone *et al.* [33], who reported that beetroot-treated samples showed slower microbial growth than controls, particularly under vacuum packaging conditions. The extract effectively reduced the total number of aerobic bacteria in the meat samples. Furthermore, antimicrobial efficacy was enhanced when beetroots were dissolved in water rather than used as a powder. In addition, it improved lipid stability and preserved sensory properties, such as color and freshness during storage.

Although vacuum-stored meat showed lower bacterial and coliform counts (Tables 1 and 2), which is favorable for its growth, particularly after the 18th day of storage. This is in line with the results of Sauvala *et al.* [35], who reported that vacuum storage of meat significantly impacts its bacterial quality by creating an anaerobic environment that suppresses the growth of aerobic spoilage bacteria, while favoring the proliferation of anaerobic or facultative anaerobic bacteria, particularly lactic acid bacteria (LAB), which dominate the microbiota under vacuum conditions and produce organic acids, leading to a reduction in pH, which can inhibit the growth of pathogenic bacteria. However, Luzardo *et al.* [36] concluded that prolonged vacuum storage can also result in spoilage due to the accumulation of metabolic byproducts from LAB, such as lactic acid and volatile compounds, which cause sour-off-odors and changes in meat color and texture. Furthermore, Sauvala *et al.* [35] showed that microbial counts, particularly mesophilic aerobic bacteria (MAB) and LAB, increased significantly after three weeks of vacuum storage at 4°C, correlating with spoilage indicators, such as pale color and unpleasant odors.

5. Conclusion

Based on the current results, beetroot extract showed a promising impact on the keeping quality and shelf-life of beef meat during refrigeration storage. The antibacterial effect of beetroot treatment was significantly affected by the application dose, where higher concentrations resulted in higher antibacterial effects. In addition, anaerobic storage extends had more powerful effect on the shelf life with maximization of the beetroot antibacterial effect. More future research efforts may be directed to assess the mechanism of beetroot extract treatment on the sensory and oxidative stability of meat and meat products.

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