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Antibacterial efficiency of apple vinegar marination on beef-borne *Salmonella*

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ABSTRACT

Background: *Salmonella*-related foodborne illnesses are a significant public health concern. Naturally, antibacterial food components have been shown to limit microbial growth proliferation with various degrees of efficacy.

Aims: To examine the occurrence, microbial load, and effect of apple vinegar on *Salmonella* serovars in beef and beef products.

Methods: 150 beef and beef products were collected between March and May 2022. Total viable count (TVC), Enterobacteriaceae count (ENT), isolation and identification of *Salmonella*, and their virulence factors detection by multiplex PCR were determined, and an experimental study of the effect of natural apple vinegar marination on *Salmonella* spp.

Results: TVC was higher in meatballs ($3.32 \times 10^6 \pm 1.07 \times 10^6$) while beef burgers ($4.22 \times 10^3 \pm 0.71 \times 10^3$) had the highest ENT. Concerning the prevalence of *Salmonella* spp., meatball (46.7%) and beef burger (25.3%) samples were the highest contamination rate. The common serovars detected were *Salmonella typhimurium* (6%), *Salmonella enteritidis* (6%), and *Salmonella infantis* (4%). Based on the results of PCR, 12, 11, and 11 out of 18 samples of *Salmonella* isolates possess *hila, stn,* and *invA* genes. By immersing the inoculated steak meat in apple vinegar at different concentrations (50%, 70%, and 100%), the initial populations of the *Salmonella* strains after 12 hours were reduced to $0.38 \times 10^2 \pm 0.05 \times 10^2$ log CFU/ml; however, after 48 hours become the most reduction ($0.31 \times 10^2 \pm 0.07 \times 10^2$ log CFU/ml) at a concentration of 100% apple vinegar. An enhancement in the sensory attributes was noted across all concentrations.

Conclusion: The consumed beef and beef products are contaminated with pathogenic bacteria such as *Salmonella* spp. Marinades made using apple vinegar concentrations of 50%, 75%, and 100% effectively minimized the prevalence of artificially inoculated *Salmonella* and extended the shelf life of preserved refrigerated beef products to 48 hours.

Keywords: Beef products*, Salmonella,* Apple vinegar, Foodborne diseases.

Introduction

Meat has a high nutritional value and is nutrient-dense due to the protein, fat, vitamins, and minerals it contains, all of which are essential for a healthy diet. Consumer awareness has elevated the importance of quality in meat and meat products (Fencioglu *et al.*, 2022). Because of its high protein and moisture content, meat is one of the most perishable foods during preparation or storage. It is subject to spoiling for various reasons, including microbial contamination (Lei *et al.*, 2023). Extensive study has been conducted into multiple processing technologies as the food sector works to ensure food safety in the face of dangerous bacteria such as *Salmonella* spp. (Cap *et al.*, 2020).

One of the major significant foodborne illnesses that can cause serious human infections and substantial economic losses worldwide is *Salmonella* spp. Their monitoring at several stages, especially in processed, unprocessed, and ready-to-eat products, acts as a benchmark for consumer safety and is an essential tool for putting actual food safety systems in place (Tîrziu *et al.*, 2020). Interest has shifted in recent trends toward the application of various usual alternatives to lengthen the duration of preservation of meat and its products and increase the storage life, especially with a significant increase in the manufacture of meat products as well as their role in providing the desired flavor and taste, many studies have been conducted to discover new

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ways to extend the period of preservation of meat and its products while avoiding chemical additives (Yagnik *et al.,* 2018; Sengun *et al*., 2019; Al-Hadidy *et al*., 2023).

Apple vinegar is one of the most well-known chemicals since it includes bioactive components with antimicrobial activities (Bhebhe *et al.*, 2022). According to Alagoz *et al.* (2020), the fermented product apple vinegar is classified as a useful food because it contains compounds and nutrients such as minerals and vitamins as well as having an inhibitory effect on many microorganisms by preventing the transport of nutrients through their cell membrane. In meat marinated in acidic marinades, a number of factors, such as acid type, acid concentration, temperature, marinating, storage duration, and initial pathogen population, may affect the growth, survival, or inactivation of *Salmonella* (Lytou *et al.*, 2019). This research aimed to investigate the microbiological quality of beef and its products and the effect of apple vinegar marination on beef-borne *Salmonella*.

Materials and Methods

Sample collection and preparation

During March-May 2022, a total of 150 beef and beef products samples, including chilled beef meats, basterma, hot dogs, meatballs, and beef burgers (30 for each), were collected from various shops, packed, identified, and transferred to the laboratory as soon as possible in an icebox to assess the microbial load and discovery of beef-borne *Salmonella* holding them. Samples weighing 25 g were placed aseptically into a sterile stomacher bag, which was then filled with 225 ml of 0.1% sterile buffer peptone water. The stomacher was then homogenized for 2 minutes at room temperature (Stomacher® 400 Circulator, Seward, Ltd., UK) to achieve a 10^{-1} dilution. In sterile distilled water, homogenized microbial samples were serially diluted. Every diluted 1 ml sample was spread out evenly on different plates.

Microbiological analysis

The plate method was used to conduct the microbiological assessment per the recognized food microbiology guidelines. Total viable count (TVC) was assessed on nutritional agar (CM0003B, Oxoid) following ISO 4833-1 (2013). On MacConkey agar (CM0115, Oxoid), the Enterobacteriaceae count (ENT) was determined following ISO 21528-2 (2017). Each analysis was done in triplicate, and standardized plate count methodologies were used to calculate plate counts and convert them to $\log_{10}$ CFU values (Vanderzant and Splittstoesser, 1992).

Salmonella isolation and identification

Each sample was placed in a stomacher bag containing 90 ml of buffered peptone water (CM0509, Oxoid) and homogenized for 1 minute (400 Circulator, Seward, London, UK) before being incubated at 37°C for 24 hours, in accordance with ISO 6579-1 (2017). Transferring 0.1 ml of nonselective pre-enrichment to 10 ml of Rappaport-Vassiliadis broth (CM0866, Oxoid) and incubating at 42°C for 24 hours. A loopful of the selective enrichment broth was streaked on xylose lysine deoxycholate agar (XLD) (CM0469, Oxoid) agar and incubated for 24 hours at 37℃. *Salmonella* colonies that exhibited a red coloration with black centers on XLD agar were identified both morphologically and biochemically, as described by Quinn *et al.* (2002). Serologically, *Salmonella* isolates were serologically identified using the Kauffman-White-Le Minor system (Grimont and Weill, 2007) to detect Somatic (O) and Flagellar (H) antigens with *Salmonella* antiserum (DENKA SEIKEN Co., Japan).

Multiplex polymerase chain reaction

Salmonella invasion (*invA*), *Salmonella* enterotoxin (*stn*), and hyper-invasive locus (*hilA*) genes are detected using multiplex PCR. DNA is extracted and purified directly from meat product samples using the QIAamp DNA Mini Kit (Cat. no. 51304), which is applied to achieve purification. Emerald Amp GT PCR master mix (Takara), RR310A code. Table 1 lists the primer sequence, target genes, and amplicon sizes. The thermocycling settings were 94°C for 2 minutes, then 30 cycles of 94°C for 45 seconds of denaturation, 53°C for 60 seconds of annealing, 72°C for 60 seconds of elongation, and 72°C for 7 minutes of final extension. The PCR products were identified by electrophoresis on a 1.5% agarose gel (Fermentas, USA) at 100 V for 30 minutes stained with SYBR SAFE (0.6 g/100 ml), and photographed with a UV transilluminator (Bio-Rad).

Table 1. The specific sequences and amplified products of oligonucleotide primers of *Salmonella* spp.

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
invA (F)	TATCGCCACGTTCGGCAA	275	Nayak *et al*. (2004)
invA (R)	TCGCACCGTCAAAGGAACC		
stn (F)	TTGTGTCGCTATCACTGGCAACC	617	Murugkar *et al*. (2003)
stn (R)	ATTCGTAACCCGCTCTCGTCC		
hilA (F)	CGGAAGCTTATTTGCGCCATGCTGAGGTAG	854	Castro *et al*. (2002)
hilA (R)	GCATGGATCCCCGCCGGCGAGATTGTG		

Effect of natural apple vinegar marination on Salmonella spp.

Preparation of apple cider vinegar

Following Al-Hadidy *et al.* (2023), place the clean apple pieces in a glass bottle, cover it with a porous cloth, place it in a warm area and let it work for a few weeks for spontaneous or self-fermentation by the naturally present yeasts *Saccharomyces cerevisiae* yeast to produce ethanol alcohol with a concentration of 11%–13%, which is known as alcoholic fermentation and followed by acetic fermentation, where the resulting alcohol turns into vinegar, known for its distinctive smell and taste. Stinging by the action of *Acetobacter* bacteria that grow on the surface of the alcoholic solution and work to oxidize it to acetic acid in the presence of oxygen, the solution is filtered to obtain vinegar and then kept in bottles sealed until use.

Fifty beef steak meat from five 2-year-old Ox at 24 hours after slaughter (in post rigor phase) were brought in refrigerated condition to the lab, cut into 2 cm thick, 10 cm long slices weighing 100 g, submerged in 70% ethyl alcohol for 3–5 minutes, and then allowing them to dry and kept at 4°C till use. With some minor modifications, *Salmonella* strains used in the current investigation that were positive for both the *invA, stn,* and *hilA* virulence genes were used in the experimental trial, as Elbarbary and Abdelmotilib (2023) stated.

Five groups of beef steak meat were used (each 10 samples). Four groups were infected with about one ml of *Salmonella* strains broth that had been adjusted to 0.5 McFarland. The inoculated steaks were saved at 37°C for 60 minutes. The first treatment was prepared by immersing the steak meat in distilled water (T1), the second treatment only got a microorganism inoculation (T2), the third treatment was prepared by immersing the inoculated steak meat in apple vinegar at a concentration of 50% (T3), the fourth treatment was prepared by immersing the inoculated steak meat in apple vinegar at a concentration of 70% (T4), and the fifth treatment was prepared by immersing the inoculated steak meat in apple vinegar at a concentration of 100% (T5). Distilled water was added to pure vinegar to produce marinades adjusted. To investigate the antimicrobial effect of apple vinegar against *Salmonella* strains, the total *Salmonella* count (ISO 6579-1, 2017) was calculated at zero, 12, 24, and 48 hours in triplicate. The findings existed as mean values and SE.

Effect of natural apple vinegar marination on organoleptic properties

Ten panelists completed an assessment form specifically designed for this purpose, utilizing a five-point hedonic scale to rate the sensory qualities of taste, color, texture, smell, and overall approval using the methodology described by Mojaddar *et al.* (2018).

Statistical analysis

The achieved results exist as a mean and standard error using the computer software program (SPSS, 2001), with a significance level of $p \leq 0.05$.

Ethical approval

Meat products were used in this study; hence, ethical agreement was not required.

Results

The information obtainable in Table 2 demonstrates the variations in the TVC (CFU/g) observed in the inspected samples. There were significant variations across the samples ($p < 0.05$). TVC was higher in meatballs ($3.32 \times 10^6 \pm 1.07 \times 10^6$) followed by beef burger ($4.8 \times 10^5 \pm 1.4 \times 10^5$), chilled beef meat ($0.75 \times 10^4 \pm 0.13 \times 10^3$), basterma ($2.37 \times 10^3 \pm 1.22 \times 10^3$), and hot dog ($1.05 \times 10^3 \pm 0.11 \times 10^2$).

Table 3 shows the ENT (CFU/g) in the inspected products were $0.18 \times 10^2 \pm 0.01 \times 10^2$, $0.34 \times 10 \pm 0.01 \times 10$, $2.38 \times 10^2 \pm 0.26 \times 10^2$, $5.18 \times 10^2 \pm 0.53 \times 10^2$, and $4.22 \times 10^3 \pm 0.71 \times 10^3$ for chilled beef meat, basterma, hot dog, meatball, and beef burger samples, respectively.

Table 4 proves the prevalence of *Salmonella* spp. (CFU/g). The studied products were 23.3%, 3.3%, 16.7%, 16.7%, 46.7%, and 25.3% of chilled beef meat, basterma, hot dog, meatball, and beef burger samples, respectively. 43.3%, 96.7%, 83.3%, 63.3%, 53.3%, and 68% of samples accepted according to EOS (2005a–d).

Table 5 demonstrates the serological identification of *Salmonella* spp. in the inspected products in which the most detected species were *Salmonella typhimurium*

Table 2. TVC (CFU/g) and their acceptability in the examined products.

Product	No.	Mean ± SE	Accepted samples		
			EOS[a]	No.	%
Chilled beef meat	30	$0.75 \times 10^4 \pm 0.13 \times 10^{3a}$	$<10^6$	26	86.7
Basterma	30	$2.37 \times 10^3 \pm 1.22 \times 10^{3b}$	$<10^4$	21	70
Hot dog	30	$1.05 \times 10^3 \pm 0.11 \times 10^{2b}$	$<10^4$	18	60
Meatball	30	$3.32 \times 10^6 \pm 1.07 \times 10^{6c}$	$<10^6$	12	40
Beef burger	30	$4.8 \times 10^5 \pm 1.4 \times 10^{5d}$	$<10^5$	11	36.7

[a]EOS: Egyptian Organization for Standardization No. (3602/2013) for chilled beef meat, (1042/2005) for basterma, (3492/2005) for hotdogs, (ES1973/2005) for meatballs, and (ES1688/2005) for beef burgers. There were significant variations across the samples ($p \leq 0.05$); mean values with the same letters in each column are not significantly different ($p \leq 0.05$).

Table 3. ENT (CFU/g) and their acceptability in the examined products.

Product	No.	Mean ± SE	Accepted samples		
			EOS[a]	No.	%
Chilled beef meat	30	$0.18 \times 10^2 \pm 0.01 \times 10^{2a}$	0	8	26.7
Basterma	30	$0.34 \times 10 \pm 0.01 \times 10^{a}$	0	27	90
Hot dog	30	$2.38 \times 10^2 \pm 0.26 \times 10^{2b}$	$<10^2$	21	70
Meatball	30	$5.18 \times 10^2 \pm 0.53 \times 10^{2c}$	0	0	0
Beef burger	30	$4.22 \times 10^3 \pm 0.71 \times 10^{3d}$	$<10^2$	0	0

[a]EOS: Egyptian Organization for Standardization No. (3602/2013) for chilled beef meat, (1042/2005) for basterma, (3492/2005) for hotdogs, (ES1973/2005) for meatballs, and (ES1688/2005) for beef burgers. nd, not detected. There were significant variations across the samples ($p \leq 0.05$); mean values with similar letters in each column do not differ significantly ($p \leq 0.05$).

Table 4. Prevalence of *Salmonella* spp. (CFU/g) and their acceptability in the examined products.

Product	No.	Positive samples		Accepted samples		
		No.	%	EOS[a]	No.	%
Chilled beef meat	30	7	23.3	0	13	43.3
Basterma	30	1	3.3	0	29	96.7
Hot dog	30	5	16.7	0	25	83.3
Meatball	30	11	36.7	0	19	63.3
Beef burger	30	14	46.7	0	16	53.3
Total	150	38	25.3	-	102	68

[a]EOS: Egyptian Organization for Standardization No. (3602/2013) for chilled beef meat, (1042/2005) for basterma, (3492/2005) for hotdogs, (ES1973/2005) for meatballs, and (ES1688/2005) for beef burgers.

(6%), *S. Haifa* (1.3%), *Salmonella heidelberg* (1.3%), *Salmonella tsevie* (2%), *Salmonella infantis* (4%), *Salmonella enteritidis* (6%), *Salmonella alfort* (0.67%), *Salmonella virchow* (1.3%), *Salmonella inganda* (2%), and *Salmonella wingrove* (0.67%).

Table 6 reveals the effect of apple vinegar on *Salmonella* count after different concentrations. The findings display that only marinating by apple vinegar at a concentration of 100% reduced the growth of *Salmonella* spp. until 24 hours from $0.58 \times 10^2 \pm 0.001 \times 10^2$ to $0.31 \times 10^2 \pm 0.07 \times 10^2$.

Table 7 shows the organoleptic properties of beef steak soaked in apple cider vinegar in different concentrations.

Figure 1 shows Multiplex PCR agarose gel electrophoresis of virulence genes of Salmonella spp., out of 18 Salmonella isolates examined, 12 were positive for hilA, 11 were positive for both stn and invA genes.

Discussion

Meat products are becoming more popular because they are quick and easy to make and resolve the fresh meat issue that is hard to find and expensive. Meat products may be made from raw ingredients that are low in microbial contamination, but they can still be contaminated during production, transportation, air, soil, operators of food, or implements. Incompletely clean conditions during production steps such as packaging, storage, and marking can also cause contamination (Younes *et al.*, 2019).

The TVC and ENT can be used to assess bacterial contamination and hygienic practices used during meat processing (Younes *et al.*, 2019). In the current investigation (Table 2), the meatball samples had the higher TVC ($3.32 \times 10^6 \pm 1.07 \times 10^6$), while the hot dog samples recorded the lowest count ($1.05 \times 10^3 \pm 0.11 \times 10^2$) may be due to vacuum packing since most spoilage microorganisms are mainly aerobic, exclusion of air inhibits the microbial growth. The differences in the count among examined samples were considered significant at ($p \leq 0.05$). According to EOS 2005a–d, 86.7%, 70%, 60%, 40%, and 36.7% of chilled beef meat, basterma, hot dog, meatball, and beef burger samples are considered acceptable depending on their TVC count. The counts were thought to be lower than those reported by Hamed *et al.* (2015) and Younes *et al.* (2019) and higher than those counts recorded by Shaltout *et al.* (2016), Ragab *et al.* (2022), and Elbarbary *et al.* (2023). The higher microbial load in beef products is probably due to the different contaminated raw materials and ingredients used and the processing methods (Elbarbary *et al.*, 2023).

Enterobacteriaceae are considered indicator microorganisms for determining meat hygiene. It is evident from Table 3 that the beef burger ($4.22 \times 10^3 \pm 0.71 \times 10^3$) samples had the most significant ENT

Table 5. Serological identification of *Salmonella* spp. in the examined products.

		O	H	No	%	Chilled meat		Basterma		Hot dog		Meatball		Beef burger	
						No	%	No	%	No	%	No	%	No	%
Salmonella typhmurim	C_2	1, 4, 5, 12	i: 1, 2	9	6	2	3.3	0	0	1	3.3	2	6.7	4	13.3
Salmonella haifa	C_1	1, 4, 5, 12	r: 1, 2	2	1.3	1	3.3	0	0	0	0	0	0	1	3.3
Salmonella heidlberg.	B	1, 4, 12	z10: 1, 2	2	1.3	0	0	0	0	0	0	1	3.3	1	3.3
Salmonella tsevie	B	6, 8	c: 1, 2	3	2	0	0	0	0	1	3.3	1	3.3	1	3.3
Salmonella infantis	E_1	6, 7	r: 1, 5	6	4	1	3.3	0	0	1	3.3	2	6.7.	2	6.7
Salmonella enteritisdis	B	1, 9, 12	g, m: 1, 2	9	6	2	3.3	1	3.3	1	3.3	3	10	2	6.7
Salmonella alfort	B	3, 10	f, g: e, n,x	1	0.67	0	0	0	0	0	0	0	0	1	3.3
Salmonella virchow	B	6, 7	z10: 1, 5	2	1.3	0	0	0	0	0	0	1	3.3	1	3.3
Salmonella inganda	C_1	6, 7, 14	r: 1,2	3	2	1	0	0	0	1	3.3	0	0	1	3.3
Salmonella wingrove	D_1	1, 4, 12	i: e, n, z15	1	0.67	0	0	0	0	0	0	1	3.3	0	0

Table 6. Effect of apple vinegar on *Salmonella* count after different exposure times.

Treatment	Exposure time (Mean ± SE)			
	Zero	12 hours	24 hours	48 hours
T1	$0.74 \times 10^2 \pm 0.01 \times 10^{2a}$	$1.85 \times 10^2 \pm 0.03 \times 10^{2a}$	$2.73 \times 10^2 \pm 1.62 \times 10^{2a}$	$2.47 \times 10^3 \pm 1.62 \times 10^{3a}$
T2	$3.15 \times 10^2 \pm 1.3 \times 10^{2b}$	$4.22 \times 10^2 \pm 1.6 \times 10^{2b}$	$3.64 \times 10^3 \pm 1.2 \times 10^{3b}$	$4.2 \times 10^3 \pm 1.27 \times 10^{3b}$
T3	$2.86 \times 10^2 \pm 1.01 \times 10^{2c}$	$2.22 \times 10^2 \pm 1.05 \times 10^{2c}$	$1.83 \times 10^2 \pm 1.2 \times 10^{2c}$	$1.8 \times 10^2 \pm 0.8 \times 10^{2c}$
T4	$2.74 \times 10^2 \pm 0.01 \times 10^{2c}$	$2.13 \times 10^2 \pm 1.1 \times 10^{2c}$	$1.46 \times 10^2 \pm 1.4 \times 10^{2c}$	$1.42 \times 10^2 \pm 0.67 \times 10^{2c}$
T5	$0.58 \times 10^2 \pm 0.001 \times 10^{2d}$	$0.38 \times 10^2 \pm 0.05 \times 10^{2d}$	$0.34 \times 10^2 \pm 0.07 \times 10^{2d}$	$0.31 \times 10^2 \pm 0.07 \times 10^{2d}$

Different superscript letters indicate that values within the same column are significantly different.
T1: immersing the steak meat in distilled water, T2: the steak meat only got a microorganism inoculation, T3: immersing the inoculated steak meat in *apple vinegar* at a concentration of 50%, T4: immersing the inoculated steak meat in apple vinegar at a concentration of 70%, T5: immersing the inoculated steak meat in apple vinegar at a concentration of 100%.

Table 7. Effect of natural apple vinegar marination on organoleptic properties of the beef steak.

Treatment	Organoleptic qualities				
	Taste	Color	Texture	Smell	General acceptance
Control (distilled water)	4^a	3^a	3^a	3^a	3^a
50% concentration	5^b	4^b	5^b	4^b	5^b
70% concentration	4^c	4^b	4^c	4^b	4^c
100% concentration	3^d	4^b	4^c	3^c	3^a

Different superscript letters indicate that values within the same column are significantly different.

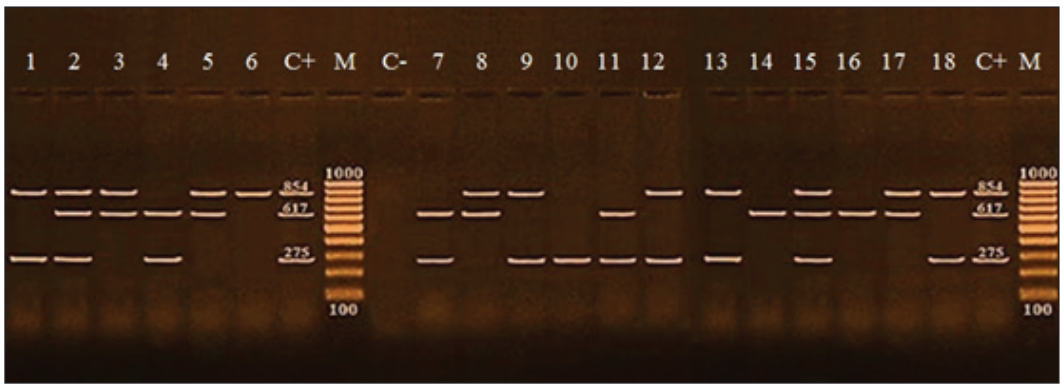

Fig. 1. Agarose gel electrophoresis of multiplex PCR of virulence genes to characterize *Salmonella* spp. M: 100 bp ladder as molecular size DNA marker. C+: control positive for *invA*, *stn*, and *hilA* genes. C-: Control negative. Lane 1, 2, 3, 5, 6, 8, 9, 12, 13, 15, 17, and 18: positive for *hilA* (854 bp). Lane 2, 3, 4, 5, 7, 8, 11, 14, 15, 16, and 17: positive for *stn* (617 bp). Lane 1, 2, 4, 7, 9, 10, 11, 12, 13, 15, and 18: positive for *invA* (275 bp).

(CFU/g) among the examined samples, while chilled beef meat ($0.18 \times 10^2 \pm 0.01 \times 10^2$) was the lowest count. In addition, there was a statistically significant link ($p \leq 0.05$) among the various sample types and the ENT. According to EOS 2005a–d, 26.7%, 90%, and 70% of chilled beef meat, basterma, and hot dog samples, respectively, were considered acceptable depending on their ENT, while all the samples of the analyzed meatball and beef burger were deemed unacceptable. These results agreed with the finding of Morshdy *et al.* (2019) but lower than those counts were recorded by Elbarbary *et al.* (2023). Foodborne illnesses caused by *Salmonella* are a significant public health concern (Morshdy *et al.*, 2019).

Overall, 38 (25.3%) samples tested positive for *Salmonella* (Table 4). Meatballs (46.7) and beef burgers (25.3%) had the highest rate of contamination; meanwhile, 68% of samples were accepted, according to EOS 2005a–d. These results concurred with those that were documented by Elsisy and Salwa (2019) but differed from those of Younes *et al.* (2019), Abdel-Atty *et al.* (2023), and Elbayoumi *et al.* (2023). Meat cutting and contamination, elevated water and oxygen concentrations, worker hand contamination, and grinder contamination are all potential sources of *Salmonella* contamination in meat products (Younes *et al.*, 2019).

Salmonella infection is among the most prevalent enteric bacterial sicknesses affecting humans. Its significance to public health is primarily related to foodborne infection, typically caused by consuming meat frequently colonized by zoonotic serovars (Melo *et al.*, 2021). Based on serotyping analyses (Table 5), 38 *Salmonella* isolates, with one isolate from each serotype. The major isolated serovars were *S. typhimurium* (6%), *S. enteritidis* (6%), *and S. infantis* (4%). The finding of the present investigations matched with the findings of Yang *et al.* (2019), Abd El-Tawab *et al.* (2020), and Melo *et al.* (2021). The most frequently reported serovars linked to human salmonellosis cases globally are *S. enterica* subsp. *enterica* serovars *enteritidis* and *typhimurium*. According to Yang *et al.* (2019), different serovars have varied disease potentials, making the determination of serotypes crucial for epidemiological surveillance and disease assessment. Therefore, the high incidence of these serovars in the current study indicates that consumers face a substantial risk. The distribution of the serovars across the various food sources suggests that *Salmonella* strains exhibit a high level of genetic variation. Other serovars detected regularly in the current investigation included *S. enteritidis*, *S. tsevie, S. inganda,* and *S. wingrove*, which have all been linked to meat contamination in other studies (Samaha *et al.*, 2016; Saad *et al.*, 2018; Abd El-Tawab *et al.*, 2020). This shows that these serovars are becoming increasingly common pollutants of food staff and should be regarded as a public health risk. Unfortunately, according to Yang *et al.* (2019), most of the *Salmonella* serovars found in the current investigation are often known to cause human salmonellosis.

DNA molecular discovery technique has recently been widely used in *Salmonella* detection, offering quick, sensitive, and specific detection. *Salmonella*'s unique virulence characteristics significantly determine its pathogenicity and contribute to the substantial morbidity and mortality associated with salmonellosis in humans (Lu *et al.*, 2022). The finding of the discovery of *Salmonella* invasive encoding genes achieved by PCR displayed in Figure 1 that there were 12 samples from 18 samples of *Salmonella* isolates which detected a *hilA* gene with a length of 854 bp, 11 samples detected a *stn* gene with a length of 617 bp, and 11 samples identified an *invA* gene with a length of 275 bp. This outcome has a similarity to that recorded by Abd El-Malek (2015), Abd El-Tawab *et al.* (2020), and Lu *et al.* (2022). Identifying many *Salmonella* virulence genes in this study allowed for some possible risk projections that will aid in public health controlling. According to

the findings of a recent study (Mthembu *et al.*, 2019; Sharma *et al.*, 2019), the delivery of *Salmonella* serovars in beef foods is not substantially associated with the sample kind ($p \leq 0.05$). The study of virulence genes can provide useful information for investigating *Salmonella* pathogenicity. The mechanism of virulence genes has to be investigated further because of the widespread occurrence of virulence genes in pathogenic bacteria and the potential for transfer. Such research will help us comprehend *Salmonella's* pathogenic mechanism while also preventing and controlling salmonellosis epidemics.

Many studies were directed to discover a new way to increase the preservation period of meat and its products and stay away from chemical additives, as attention has turned in recent years toward the use of numerous natural alternatives to extend the life of meat preservation and its products and increase the storage life, especially with a significant increase in the manufacture of meat products as well as their role in giving the desired flavor and taste, they can lengthen the shelf life of the food product because of its various therapeutic properties that cause its chemical content of active groups such as phenolic compounds and organic acids (Al-Hadidy *et al.*, 2023). Apple cider vinegar is a fermented product classified as a functional food because of its compounds and nutrients, such as minerals and vitamins in addition to having inhibitory effectiveness toward many microorganisms through its role in inhibiting the transport of nutrients through its cell membrane as well as its role in improving its manufacturing properties (Alagoz *et al.*, 2020), marinating can be described as the processing of raw meat with numerous components comprising vinegar or organic acids, oil, salt, sugar, herbs, spices and flavoring constituents for tenderizing meat, improving its juiciness and flavor, as well as improving microbiological properties and safeguarding the meat safety (Lopes *et al.*, 2022). The effect of apple vinegar on *Salmonella* count and holding time in inoculated beef steaks *in vitro* (Table 6) by immersing the inoculated steak meat in apple vinegar at different concentrations (50%, 70%, and 100%). Initial populations of the *Salmonella* after 48 hours in apple vinegar at a concentration of 50% (T3) were $1.8 \times 10^2 \pm 0.8 \times 10^2$ log CFU/ml whereas at 70% (T4) were $1.42 \times 10^2 \pm 0.67 \times 10^2$ log CFU/ml. Under the same conditions, the initial populations of the *Salmonella* strains after 12 hours were reduced to $0.38 \times 10^2 \pm 0.05 \times 10^2$ log CFU/ml in T5; however, after 48 hours became the most reduction ($0.31 \times 10^2 \pm 0.07 \times 10^2$ log CFU/ml) in apple vinegar at a concentration of 100% (T5).

In contrast, in positive control samples, *Salmonella* count was higher than initial populations at $4.2 \times 10^3 \pm 1.27 \times 10^3$ CFU/g after 48 hours. As expected, the apple vinegar was very active against *Salmonella*, especially with a high concentration (100%), and the outcomes of this investigation corroborated the conclusions of Lytou *et al.* (2017), Al-Hadidy *et al.* (2023) and Łepecka *et al.* (2023). The decrease in the number of bacteria after 48 h of preservation is evidence of the effectiveness of apple cider vinegar as a natural preservative because it contains many active compounds such as antimicrobial antagonists, as vinegar contains organic acids such as acetic and malic acid, phenolic compounds such as phenol, cresols and ketone compounds (Al-Hadidy *et al.*, 2023). Organic acids act to prevent the growth of bacteria through several methods, including the destruction of the outer membrane of bacteria, the consumption of microbe energy, and an increase in osmotic pressure causing the destruction of the cell membrane and promoting the production of antibacterial peptides in host cells, forcing them to release many essential nutrients such as glutamic and acid ions, to balance the osmotic pressure inside the cells, which leads to inhibiting the usual development of bacteria (Al-Hadidy *et al.*, 2023). The minimal use of additional substances in organic production, which is frequently a significant technological challenge, is encouraging developments in the application of apple vinegar to organic meat processing.

The sensory evaluation of beef steak with apple vinegar is shown in Table 7; for the color characteristics used in different concentrations (50%, 70%, and 100%), there is no significant difference between the groups ($p \leq 0.05$). It is believed that the acidic or oregano acid color of the fruit used may change the properties of the meat treated. It was noted that apple vinegar was a good source of increased product color. In contrast, it is noted that there is a significant difference in the grades granted to the other characteristics of the product. The results also show a decrease in the taste, smell, texture, and general acceptance values of the product with an increase in the concentration used compared to the results of the treatment control and the concentration of 50% followed by the concentration of 70% recorded the best sensory evaluation than the concentration of 100%. The results agreed with the finding of Al-Hadidy *et al.* (2023) and Łepecka *et al.* (2023). The use of vinegar has been shown to have a number of advantages, including improved meat sensory qualities, color, palatability, tenderness, increased secretion of bioactive substances from muscle tissue, microbiological safety, and increased product durability (Lopes *et al.*, 2022).

Conclusion

This research demonstrates that beef and beef products are contaminated with pathogenic bacteria such as *salmonella*. As a result, the microbiological quality of beef products must be improved to reduce consumer contamination hazards. Furthermore, marinades with apple vinegar concentrations of 50%, 75%, and 100% were found to be the most efficient in lowering the incidence of artificially inoculated *Salmonella* and increasing the shelf-life of treated refrigerated beef products by up to 48 hours without losing their sensory

properties. Once optimized, these marinades can provide consumers and producers with a low-cost and enticing method to enhance food safety.

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Conflict of interest

No conflicts of interest exist, according to the authors.

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Authors' contributions

Samples collection by Mohamed K. Dandrawy, isolation and strain characterization by Nady Khairy Elbarbary and Neveen M. Abdelmotilib, drafting and manuscript writing by Mohamed M. Salem and Soumya Singh, and Mounir M. Salem-Bekhit supervised and participated in writing the manuscript. All authors read, commented on, and approved the final manuscript.

Data availability

All data are provided in the manuscript. Any extra data needed can be provided by the corresponding author upon reasonable request.

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