Haryana Veterinarian (Dec., 2025) **64(2),** 10-15 ISSN 0033-4359 (P)

Research Article

A COMPARATIVE STUDY OF NON-THERMAL METHODS FOR REDUCTION OF ANTIMICROBIAL RESIDUES IN BUFFALO MEAT

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Received: 21.02.2025; Accepted: 03.04.2025

ABSTRACT

Antimicrobial residues in animal-derived foods pose health risks, and traditional methods are often inadequate to address these effectively. The objective of this study was to assess the effectiveness of non-thermal technologies- Infrared (IR), Ultraviolet (UV), and X-ray in reducing antimicrobial residues in buffalo meat. Buffalo meat samples spiked with six antimicrobials were treated with non-thermal methods and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). UV treatment proved the most effective, achieving up to 77.64% reduction in sulfamethazine and 68.22% in ciprofloxacin. While IR and X-ray treatments were less effective in comparison to UV treatment. Despite notable reductions, none of the methods, including infrared (IR), ultraviolet (UV), and X-ray, eliminated antimicrobial residues. Therefore, it is highlighted that UV treatment was the most promising method for better residue removal, and food safety is necessary.

Keywords: Carabeef, IR treatment, LC-MS/MS, UV treatment, Veterinary Drug Residues

How to cite: Zende, R.J., Vaidya, V.M., Shukla, V.H., Panicker, N.S., Nair, A.S., Shirke, A.H. and Tambe, S.M. (2025). A comparative study of non-thermal methods for reduction of antimicrobial residues in buffalo meat. *Haryana Veterinarian.* **64(2)**: 10-15.

Veterinary antimicrobials, although initially developed to treat and prevent infections, have been widely used in animal farms for disease control. Subsequently, the non-judicious use led to the presence of veterinary drug residues in animal-origin foods (Almashhadany *et al.*, 2022). India alone accounts for 3% of the total global consumption of antimicrobials in food-producing animals (Dhangar *et al.*, 2023). These drug residues accumulate in the liver, kidney, and even in the muscles of the food animals (Qamar *et al.*, 2023; Treiber and Beranek, 2021). It has been proposed that a key priority for public health is antibiotic resistance in microorganisms, particularly obtained in food animals (Kumar *et al.*, 2024). Several adverse effects, including immunopathological effects, nephropathy (gentamicin), bone marrow toxicity (chloramphenicol) and even carcinogenicity (sulphamethazine, oxytetracycline, furazolidone), may result from these residues (Ibarra *et al.*, 2014).

In order to monitor and control the antimicrobial residues in meat, continuous monitoring and surveillance of the samples is necessary. A potential method to control residues can be to treat the meat with non-invasive methods. Certain studies have shown a 90% reduction of the initial level of tetracycline by continuously treating chicken meat for 106.6 minutes with a thermal method like microwave (Abou-Raya *et al.*, 2013). Conventional food processing methods, however, typically cause detrimental effects on the physico-chemical properties of food products, probable nutritional loss, deterioration in the quality of the product acceptance, and customer demand (Augusto *et al.*, 2018). The demand for convenient, long-lasting, and safe foods with "fresh-like" attributes has increased in recent years. Therefore, innovations in non-thermal technologies, such as UV light, are gaining attention for their potential to kill pathogenic microorganisms while maintaining the quality of the product (Aaliya *et al.*, 2021). However, extensive studies on the action of non-thermal techniques on antimicrobial residues are scarce, presenting a vast potential for additional research.

The study aimed to evaluate the efficacy of non-thermal processing methods for reducing antimicrobial residues in buffalo meat from animals unexpectedly slaughtered before the entire withdrawal period and preserving its quality. It further focused on a comparative analysis of three non-thermal techniques-Infrared (IR), Ultraviolet-visible (UV), and X-ray radiation-assessing their effectiveness in deactivating antimicrobial residues. Based on the surveillance of veterinary medical stores and on feedback from large animal practitioners, it was revealed that six antibiotics *viz*. sulfamethazine, sulfadoxine, ciprofloxacin, enrofloxacin, oxytetracycline, and chlortetracycline, are commonly used for the treatment of various bacterial diseases in buffaloes. Additionally, the study examined the meat samples for various quality parameters before and after treatment.

MATERIALS AND METHODS

All six antimicrobials, such as sulfamethazine

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(99%), sulfadoxine (99%), ciprofloxacin (99%), enrofloxacin (99%), oxytetracycline (99%) and chlortetracycline (99%) standard were purchased from Sigma Aldrich, USA. Acetonitrile, Methanol, Water of LC-MS/MS grade and Formic acid (LC-MS LiChropur) were procured from Merck India Private Limited, USA. Oasis HLB 6 cc (200 mg) extraction cartridges were purchased from Waters India Pvt Limited, India.

Sample collection and storage:

A total of 250 buffalo meat samples (500 g) were collected from Deonar Abattoir, Mumbai, and brought in insulated containers on ice to the laboratory for further analysis. The edible portion was minced, homogenized before further testing.

Instrumental Conditions:

The LC-MS/MS analysis was performed using an ultra-high performance liquid chromatography (Waters Acquity UPLC) coupled with a triple quadrupole mass spectrometer (Waters Xevo TQ-S micro mass spectrometer, USA) operated by Mass Lynx software, version 4.2. The column ACQUITY UPLC BEH C18 1.7µm (2.1×50 mm) was used for the chromatographic separation of the targeted analytes. The mobile phases comprised: Eluent A: 0.1% formic acid in water, and Eluent B: 0.1% formic acid in methanol.

Mass spectrometry was performed using a Waters Xevo TQ-S micro mass spectrometer. The mass spectrometer used an electrospray ionization source in the positive mode (ESI+). The following optimized ion source parameters were used: desolvation temperature (600° C), cone gas (50 L/h), ion source temperature (150° C), desolvation gas (1000 L/h), and capillary voltage (1.5 kV). The optimized multiple reaction monitoring (MRM) transitions for every analyte are presented in Table 1.

Sample processing

Sample preparation was carried out according to Zhang *et al.* (2018) with slight modifications. A total of 2.50±0.01 g of homogenized sample was taken and 10 ml of 80% acetonitrile in water was added, vortexed for 10 seconds, and sonicated. Centrifugation was done at 7000 rpm for 5 min at 4° C. Then, 4 ml of an aliquot was taken for solid phase extraction and passed through the Oasis HLB 6 cc cartridge. The extracted sample was evaporated near dryness under a nitrogen evaporator and was reconstituted in 1 mL of 20% acetonitrile in water. The solution was filtered through a 0.22 µm syringe filter and transferred to an autosampler vial for LC-MS/MS analysis.

Sample analysis:

A five-point calibration curve was plotted (10, 20, 50, 100 and 150 µg/kg) in solvent as well as in the matrix extract. The linearity study was carried out by spiking blank buffalo meat at concentrations of 10, 20, 50, 100 and 150 µg/kg of antimicrobial standards prepared in methanol (v/v). The calibration graphs were evaluated with the coefficient of regression (r2) > 0.99.

Spiking of known concentration of antimicrobials in buffalo meat:

The blank sample was spiked with a mixture of six antimicrobials: sulfamethazine, sulfadoxine, ciprofloxacin, enrofloxacin, oxytetracycline, and chlortetracycline (100 µg/kg each). The primary aim was to assess the recovery of these antimicrobials after the application of non-thermal processing methods using liquid chromatography-tandem mass spectrometry (LC-MS/MS). A total of six replicates of the sample were prepared and each sample was analyzed with three LC injections of the same samples for consistent data on the recovery rates of the spiked antimicrobials. The standardization and validation of the method were conducted as per SANTE Guidelines (SANTE/12682/2019).

Exposure of non-thermal treatments on spiked buffalo meat samples:

The effect of different non-thermal treatments on the reduction of antimicrobial residues was observed using Infrared (IR), Ultraviolet (UV), and X-ray techniques. The spiked buffalo meat samples, each with a thickness of 1 cm, were uniformly placed at a distance of 21 cm (considered based on different trials conducted during the study) from the non-thermal treatment sources to ensure consistent exposure. To prevent direct contact between the meat and the non-thermal treatments, the samples were enclosed in polyethylene packaging (150 g/m^2/24 h) film. All the treated samples were then further processed for antimicrobial residue extraction and analysis by LC-MS/MS.

Infrared Treatment (IR):

These spiked buffalo meat samples were then subjected to infrared (IR) treatment using a 250-watt IR lamp for 9 minutes. The temperature during the treatment was found to be increased to 70° C.

UV treatment:

The treatment was performed in a UV cabinet, which consists of 8 UV tubes, four each of 11 and 8 watts, having wavelengths of 365 and 254 nm, respectively, for 15 minutes.

X-ray Treatment:

The samples were treated with an X-ray source, with a power output of 48 KW of X-ray for 10 seconds.

Table 1. Details of Multiple Reaction Monitoring (MRM) transitions used for standard antimicrobials

Sr.No.	Name of the analyte	Precursor Ion	Product Ion
1.	Sulfamethazine (SMZ)	279.10	124.10, 186.00
2.	Sulfadoxine (SDX)	311.01	92.00, 156.00
3.	Ciprofloxacin (CIP)	332.10	288.10, 314.10
4.	Enrofloxacin (ENR)	360.30	316.30, 342.30
5.	Chlortetracycline hydrochloride (CTC)	479.30	444.184, 462.191
6.	Oxytetracycline hydrochloride (OTC)	460.65	426.216, 444.226

Table 2. Percent recovery of antimicrobial residue concentrations of buffalo meat samples spiked at 100 µg/kg

Replicates	Percent recovery of antimicrobial residues (µg/kg)					
	Sulfamethazine	Sulfadoxine	Ciprofloxacin	Enrofloxacin	Oxytetracycline	Chlortetracycline
1	88.53±2.37	88.13±2.56	106.13±5.66	72.60±0.70	121.43±28.37	87.67±0.78
2	83.30±1.64	81.30±1.71	97.13±2.80	68.97±1.31	96.73±31.76	84.73±0.90
3	84.23±1.96	84.43±1.55	98.93±1.91	67.50±1.10	98.80±19.00	83.23±2.29
4	78.10±2.27	79.07±2.15	93.20±3.08	61.33±0.55	98.80±36.41	76.63±0.21
5	87.27±2.16	86.80±2.18	101.13±3.42	64.13±1.60	113.30±54.39	84.27±3.52
6	88.90±0.62	93.67±1.69	107.50±1.22	61.20±0.79	112.10±6.17	81.77±0.81
Avg.	**85.06±4.09^{b}**	**85.57±5.20^{b}**	**100.67±5.44^{a}**	**65.96±4.53^{c}**	**110.19±14.86^{a}**	**83.05±3.70^{b}**

*Different letters are significantly different.

Table 3. Percent Reduction of antimicrobial residues in non-thermally treated buffalo meat samples.

Antimicrobial	Percent Reduction of antimicrobial residues using different non-thermal treatments		
	IR	UV	X-Ray
Sulfamethazine	42.59±15.35^{bc}	77.64±5.94^{a}	28.13±3.86^{b}
Sulfadoxine	37.20±13.98^{e}	65.64±7.52^{d}	22.34±3.88^{d}
Ciprofloxacin	26.70±18.98^{f}	68.22±7.89^{bc}	13.72±6.17^{e}
Enrofloxacin	51.34±13.87^{a}	63.59±6.41^{e}	30.27±4.31^{a}
Oxytetracycline	39.31±9.18^{d}	69.46±5.94^{b}	27.23±23.24^{bc}
Chlortetracycline	43.20±15.21^{b}	65.81±3.69^{d}	15.70±4.06^{e}

*Different letters are significantly different.

Table 4. Physico-chemical parameters of non-thermally treated buffalo meat samples

Treatments	pH	Color (ΔE)	TBA (mg MDA/kg)	Tyrosine (g/kg)
Control	5.35±0.03^{a}	0.32±0.12^{e}	0.077±0.002^{e}	0.324±0.002^{d}
IR	5.01±0.02^{bc}	7.77±0.01^{b}	0.114±0.002^{c}	0.381±0.007^{b}
UV	4.96±0.02^{c}	7.35±0.12^{c}	0.130±0.001^{b}	0.384±0.004^{b}
X-Ray	5.08±0.01^{b}	6.40±0.09^{d}	0.094±0.002^{d}	0.357±0.002^{c}

*Different letters are significantly different.

Quality parameters of pre- and post-radiation treated meat samples:

Physico-chemical analysis of all the buffalo meat samples (pH, color (ΔE), TBA and tyrosine) before and after exposure to non-thermal methods was performed.

RESULTS AND DISCUSSION

A control group of samples spiked with a known concentration of antimicrobials (100µg/kg) was not subjected to any non-thermal treatment. The calibration curve of spiked buffalo meat showed the coefficient of regression (r^2) ≥ 0.99. The recovery concentration of oxytetracycline and ciprofloxacin was found to be highest in the control sample, while enrofloxacin was lowest (Table 2).

IR-treated buffalo meat samples:

The antimicrobials obtained from buffalo meat treated with IR are depicted in Table 3. It was observed that the highest % reduction of antimicrobial residues in IR-treated meat samples was in enrofloxacin, followed by chlortetracycline, sulfamethazine, oxytetracycline, sulfadoxine and ciprofloxacin compared to control samples. This supports the hypothesis that fluoroquinolones are particularly susceptible to IR-induced degradation due to their chemical structure, which can be facilitated by changes in pH or light (Bairros *et al.*, 2018). The IR treatment may have contributed to the breakdown of chlortetracycline, possibly by enhancing the chemical reactions that lead to its degradation (Huang *et al.*, 2019).

UV-treated buffalo meat samples:

The buffalo meat samples treated with UV light showed the lowest sulfamethazine levels, while ciprofloxacin and oxytetracycline were the highest compared to the control sample. In UV treatment, the highest % reduction of antimicrobial residues was observed for sulfamethazine, followed by oxytetracycline, ciprofloxacin, chlortetracycline, sulfadoxine and enrofloxacin compared to control samples (Table 3). The remarkable reduction in antimicrobial residues is in agreement with previous studies, which

indicates the effect of UV light in breaking down chemical compounds, including antimicrobials (Keen *et al.*, 2013). UV light promotes photodegradation by generating reactive oxygen species (ROS), such as hydroxyl radicals, which degrade the antimicrobials (Lu *et al.*, 2022; Han *et al.*, 2023). This explains the pronounced reduction in sulfamethazine residues compared to other antimicrobials.

X-ray-treated buffalo meat sample:

It is observed that the buffalo meat samples exposed to X-ray showed the highest ciprofloxacin residue, whereas sulfamethazine showed the lowest. In X-ray treatment, the highest % reduction of antimicrobial residues was observed for enrofloxacin, followed by sulfamethazine, oxytetracycline, sulfadoxine, chlortetracycline and ciprofloxacin compared to control samples (Table 3).

Comparison of the Efficiency of non-thermal treatments on antimicrobial residues:

The % reduction of antimicrobial residue in buffalo meat samples treated with IR, UV and X-ray indicates that the highest % reduction was observed in the residual concentration of sulfamethazine (77.64±5.94 %), whereas the lowest percent reduction was observed in the residual concentration of ciprofloxacin (13.72±6.17 %) in buffalo meat samples (Table 3).

In the present study, the UV treatment showed a maximum reduction of all the antimicrobials compared to IR and X-ray treatments, owing to the longer exposure duration. UV treatment was more effective in reduction (%) of all the antimicrobials and followed the following trend: sulfamethazine > oxytetracycline > ciprofloxacin > chlortetracycline > sulfadoxine > enrofloxacin. X-ray treatment showed less reduction (%) in all the antimicrobials and followed the following trend: enrofloxacin > sulfamethazine > oxytetracycline >sulfadoxine > chlortetracycline > ciprofloxacin. None of the non-thermal treatments was able to eliminate all six antimicrobial residues from buffalo meat samples.

Physico-chemical analysis of non-thermal-treated buffalo samples:

The physico-chemical properties of buffalo meat were significantly influenced by non-thermal processing (Table 4). A decrease in the pH of the treated samples was observed as compared to the control (5.35±0.03). Similar trends have been observed in previous studies, where such treatments resulted in lower pH values due to the degradation of proteins and release of acidic by-products (Shija *et al.*, 2013). Whereas, a significant increase in ΔE colour values from 0.32±0.12 in the control to 7.77±0.01 in the treated samples was observed. These changes may be primarily observed due to metmyoglobin formation caused by myoglobin oxidation, leading to a darker colour in meat products (Bekhit *et al.*, 2013; Lopes *et al.*, 2016). Moreover, the rise in TBA values (0.077±0.002 to 0.130± 0.001 mg MDA/kg) suggests an increase in lipid peroxidation during non-thermal processing. This is consistent with findings from similar studies where non-thermal processing methods showed increased lipid oxidation due to prolonged exposure durations (Jayasena *et al.*, 2015). Additionally, an increase in the tyrosine content from 0.324±0.002 to 0.384±0.004 g/kg was observed in the samples. An increase in the tyrosine content reflects the breakdown of proteins, which may enhance certain sensory attributes, such as flavour, but can also lead to decreased textural integrity, depending on the processing intensity (Khalid *et al.*, 2023).

CONCLUSION

The present study introduces an innovative approach to reduce residues without significantly compromising the texture or overall quality of the meat, providing a potentially sustainable and non-invasive alternative to conventional methods to tackle antimicrobial contamination in the food supply. However, this investigation of the effect of non-thermal treatment on antimicrobial residues was studied at the laboratory level with a smaller sample size. There is scope for further studies to be carried out with a large number of samples to identify the effect of non-thermal treatment on antimicrobial residue reduction at the commercial level.

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