

## QUALITY AND SAFETY EVALUATION OF CHICKEN SHAWARMA IN CHENNAI

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### ABSTRACT

*Shawarma is a popular ready-to-eat food consumed by a diverse spectrum of populations in Chennai and other parts of India. The consumption of shawarma has dramatically increased in recent years. Although it is consumed by many people, especially the younger generation, the quality and safety of the shawarma are questionable. In this study, 10 chicken shawarma samples were purchased from local restaurants in Chennai city to assess their quality and safety by estimating rancidity profile in terms of free fatty acid (FFA) and peroxide value, histamine content, and microbial contamination. The results revealed that the percentage of FFA and peroxide values ranged from 1.41±0.99 to 7.05±1.73 and 2±0.57 to 14±1.47 meq/kg, respectively. Among the tested samples, 30% showed the presence of a significant level of histamine content (1.7–7 mg/100g). The biochemical characterization of bacterial isolates from shawarma samples revealed the presence of contamination by *Salmonella enteric sub sp. enterica*, *Escherichia coli* STEC 367, *Listeria monocytogenes*, and *Listeria innocua*. Further, these isolates were also confirmed by PCR by amplifying a 284 bp *invA* gene fragment for *Salmonella* sp., an 884 bp *uspA* gene fragment for *E. coli*, and a 457 bp *iap* gene fragment for *Listeria* sp. The findings of this study emphasize the significance of safety monitoring for ready-to-eat foods.*

**Key words:** *Shawarma, rancidity profile, histamine, microbial contamination*

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### INTRODUCTION

Shawarma originated in the Ottoman Empire. It is a common snack made using ingredients such as pita or wrap bread; meat

(such as lamb, chicken, turkey, or beef); vegetables (chopped or shredded); pickles; and sauces. It is commonly consumed in regions including India, Egypt, Iraq, the Levant, the Caucasus, and Russia, and it is considered a healthy source of protein, fats, and vitamins associated with several health benefits (Weichselbaum *et al.*, 2013). However, microbial loads in ready-to-eat shawarma may be greatly increased by cross-contamination from raw meat, poor

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personal hygiene, and unsanitary practices employed by food handlers and vendors.

Unfortunately, most ready-to-eat shawarma is prepared and sold by street vendors without adherence to basic hygiene practices, putting consumers at risk of bacterial contamination that can cause gastroenteritis and other food-borne illnesses. Furthermore, shawarma can readily become contaminated by various types of microorganisms during preparation processes such as cutting, slicing, and packaging. In many agricultural countries, a large portion of the population consumes fewer calories and depends on grains or root crops; therefore, meat and meat products can be valuable additions to such diets. Street-distributed ready-to-eat meat products such as shawarma are particularly susceptible to microbial contamination, as they are prepared, served, and distributed in open or crowded environments that often lack the basic sanitary conditions required for food establishments. Microbiological loads in shawarma may be increased by several factors, including cross-contamination from raw meat, poor personal hygiene, and unsanitary practices among food handlers and vendors (Abdelhai *et al.*, 2015).

Histamine, a biogenic amine in food, is produced by bacterial decarboxylation of histidine as a result of microbial contamination, poor hygiene during preparation, improper handling, or inadequate storage conditions. The histamine content in ready-to-eat (RTE) shawarma is a crucial indicator for assessing food safety, ensuring quality, and protecting

public health from potential allergic-like food poisoning. Studies have shown that shawarma often contains histamine levels exceeding international permissible limits, which can lead to allergic-type poisoning in consumers, causing symptoms such as headaches, rashes, diarrhea, vomiting, nausea, and tachycardia, particularly in individuals with histamine intolerance or low levels of the enzyme diamine oxidase (DAO). Against this background, the present study evaluates the quality and safety of shawarma in the city of Chennai.

## MATERIALS AND METHODS

The present study was carried out at the Pharmacovigilance Laboratory for Animal Feed and Food Safety (PLAFFS), Centre for Animal Health Studies at Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai. All analyses were performed in triplicate.

### Sample source

A total of 10 Chicken shawarma samples used in the present study were purchased and collected in sterile, sealed bags under cold conditions from 10 well-known and widely distributed restaurants in the city of Chennai, Tamil Nadu. Subsequently, the samples were processed within 1 h of purchase for biochemical and microbiological characterization in accordance with the recommendations of the World Health Organization (Aidara-Kane *et al.*, 2018).

## Rancidity Profile

The rancidity profile was estimated in terms of free fatty acid (FFA) and peroxide value.

### Free fatty acid (FFA) determination

Two grams of cooked shawarma oil was titrated with 1 N KOH using phenolphthalein as an indicator until the solution became pink. If the endpoint was not observed, the oil sample was preheated at 75–80°C for 15 minutes in a water bath. The percentage of FFA was calculated using the following formula:

$$\text{FFA}\% = \frac{(\text{Titrate value} - \text{Blank value}) \times \text{Normality of KOH} \times 28.2}{\text{Weight of the sample}}$$

### Peroxide value determination

The peroxide value of the oil, which indicates its degree of oxidation and rancidity, was determined. Approximately 5 g of oil sample was added to a 250 ml conical flask containing 30 ml of an acetic acid–chloroform mixture (3:2). Then, 0.5 ml of saturated potassium iodide solution was added, and the mixture was stirred thoroughly. The mixture was kept in the dark for 1 minute and then titrated with 0.1 N sodium thiosulphate until all traces of the blue colour disappeared. Subsequently, 0.5 ml of starch was added as an indicator, and the titration was continued with 0.01 N sodium thiosulphate. A blank determination was performed using 0.1 N sodium thiosulphate. The peroxide value was expressed in milliequivalents of peroxide

oxygen per kilogram (meq/kg) using the following formula:

$$\text{Peroxide value} = \frac{\text{Titrate value} \times N \times 1000}{\text{Weight of the sample}}$$

### Microbial quality assessment

A 25 g portion of each sample was blended with 225 ml of 0.1% sterile peptone water for 3 min at high speed. Serial dilutions from 10<sup>-1</sup> to 10<sup>-6</sup> were prepared, and microbiological analyses were performed using 1 ml from these dilutions.

### Total plate count (TPC)

The total plate count (TPC) was determined for 10 shawarma samples using TPC agar. The plates were incubated at 37 °C for 24–48 h after spreading serially diluted samples on TPC agar plates. Bacterial growth was then observed and recorded.

### Yeast and mould count

The yeast and mould count was determined for 10 shawarma samples using potato dextrose agar (PDA). The plates were incubated at 28 °C for 3–5 days after spreading serially diluted samples on PDA plates. Yeast and mould growth was then observed and recorded (Bailey and Scott, 1998).

### Isolation and characterization of *Salmonella*

The isolation of *Salmonella* from shawarma samples was performed using the method described by Alharbi *et al.* (2019), with slight modifications. Pre-

enrichment of *Salmonella* was carried out by inoculating 1 ml of diluted sample into 9 ml of non-selective broth (buffered peptone water) and incubating at 37°C for 18–24 h. Subsequently, a loopful of pre-enriched culture was streaked onto XLD agar and selective *Salmonella* chromogenic agar plates. The plates were incubated at 37°C for 18–24 h, after which *Salmonella* isolates were identified.

Biochemical characterization of *Salmonella* was performed using the HiSalmonella identification kit (KB011, HiMedia) by inoculating *Salmonella* isolates obtained from XLD agar and Salmonella Chromogenic Agar selective plates. As per the manufacturer's instructions, the following tests were performed: Methyl Red test, Voges-Proskauer test, Urease test, H<sub>2</sub>S production test, Citrate Utilization test, Lysine Utilization test, ONPG test, and tests for Lactose, Arabinose, Maltose, Sorbitol, and Dulcitol.

Molecular characterization of *Salmonella* was performed by PCR amplification of the *Salmonella*-specific *invA* gene. DNA was extracted from *Salmonella* isolates using the DNeasy Blood and Tissue Kit (Qiagen, Korea) and quantified using a Nanodrop spectrophotometer (Thermo Scientific). PCR amplification of the *invA* gene was performed using a Bio-Rad Thermal Cycler. Reactions were carried out in a 25 µL volume containing 2 µL of 50 ng DNA template, 12.5 µL of Amplicon Master Mix, 1 µL of each forward (5'-GTGAAATTATCGCCACGTTTCGGGCAA-3') and reverse (5'-TCATCGCACCGT

CAAAGGAACC-3') primer and 8.5 µL of nuclease-free water. The PCR program consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of 1.5 min at 95°C, 1 min at 62°C and 1.5 min at 72°C, with a final extension at 72°C for 7 min. The amplified PCR products were electrophoresed in 1.5% agarose gel stained with 0.5 µg/ml ethidium bromide and photographed using the Bio-Rad Gel Documentation System.

### Isolation and characterization of *E. coli*

The isolation of *E. coli* isolates was performed as described for *Salmonella* isolation. Biochemical characterization of *E. coli* was conducted using the HiE.Coli™ Identification Kit (KB010, HiMedia) by inoculating *E. coli* isolates obtained from XLD agar and Salmonella Chromogenic Agar selective plates. As per the manufacturer's instructions, the following tests were performed: Methyl Red test, Voges-Proskauer test, Citrate Utilization test, Lysine Utilization test, ONPG test, and tests for Lactose, Sorbitol, Indole, Glucuronidase, Nitrate reduction, Glucose and Sucrose.

Molecular characterization of *E. coli* was performed by PCR amplification of the *E. coli*-specific *uspA* gene using forward (5'-CCGATACGCTGCCAATCAGT-3') and reverse (5'-ACGCAGACCGTAAGGGCCAGAT-3') primers. DNA isolation, PCR reaction mixtures, thermal cycler program and electrophoresis of PCR products were performed as described in the molecular characterization of *Salmonella* section.

### Isolation and characterization of *Listeria*

A 25 g portion of homogenized shawarma samples was added to 225 ml of Fraser broth enrichment medium and incubated at 37°C for 24 h. A loopful of enriched cultures was then streaked onto Oxford agar selective plates. *Listeria* isolates were identified after the plates were incubated at 37°C for 24–48 h.

Biochemical characterization of *Listeria* was performed using the HiListeria™ Identification Kit (M1327, HiMedia) by inoculating isolates obtained from Oxford agar selective plates. As per the manufacturer's instructions, the following tests were performed: Catalase, Nitrate reduction, Esculin hydrolysis, Voges-Proskauer test, Methyl Red test, Xylose, Lactose, Glucose,  $\alpha$ -Methyl-D-mannoside, Rhamnose, Sucrose and Mannitol tests.

Molecular characterization of *Listeria* was performed by PCR amplification of the *Listeria*-specific *iapA* gene. DNA isolation and electrophoresis of PCR products were carried out as described in the molecular characterization of *Salmonella* section. PCR reactions were performed in a 25  $\mu$ l volume containing 2  $\mu$ l of 50 ng DNA template, 12.5  $\mu$ l of Amplicon Master Mix, 1  $\mu$ l of each forward (5'-ATGTCATGGAATAA-3') and reverse (5'-AAAAACACCTTGAAAAGC-3') primer and 8.5  $\mu$ l of nuclease-free water. The PCR program consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of 1 min at 95°C, 2 min at 36°C and 3 min at 72°C, with a final extension at 72°C for 7 min.

### Estimation of histamine by High Performance Thin Layer Chromatography (HPTLC)

One gram of homogenized shawarma samples was added to 10 mL of methanol (chromatography grade) and centrifuged at 10,000 rpm for 10 min after thorough mixing. The supernatant was transferred to a 25 ml beaker and completely evaporated on a hot plate. The dried residue was re-suspended in 500  $\mu$ l of methanol and filtered using a 0.25  $\mu$ m nylon syringe filter. The syringe-filtered filtrates were used for HPTLC analysis using CAMAG VisionCat software. Five microliters of filtrate were applied on silica gel 60 F254 pre-coated TLC plates as 8 mm wide bands with 4 mm spacing between bands, using an automatic TLC applicator (Linomat-5) with N<sub>2</sub> gas flow at 7 psi. The chromatographic chamber (20×10 cm CAMAG twin-trough chamber) was saturated with the mobile phase (acetone:ammonia, 95:5 v/v) for 20–30 min before linear ascending development on the plate, allowing the mobile phase to migrate 70 mm. The plate was air-dried and derivatized with 3% ninhydrin spray. Densitometric scanning was performed using a CAMAG Scanner 3 with absorbance measured at 410 nm, a slit dimension of 6×0.45 mm and a scanning speed of 20 mm s<sup>-1</sup>.

### RESULTS AND DISCUSSION

A total of 10 shawarma samples were collected from local street shops in Chennai in sterile bags and stored at cold temperature.

## Rancidity Profile

*Free fatty acid (FFA) profile:* The percentage of FFA content in the tested shawarma samples ranged from  $1.41 \pm 0.99$  to  $7.05 \pm 1.73$  (Figure 1). According to FSSAI regulatory standards, the acceptable FFA content is 0.5–0.8%. In this study, all samples exceeded the FSSAI acceptable limit, and three samples showed FFA content  $>4\%$ . Similarly, Mariana et al. (2020) observed changes in FFA content in five ‘Nasi Lalapan’ samples at different time intervals during frying.

*Peroxide value (PV):* The PV content among the tested samples ranged from  $2 \pm 0.57$  to  $14 \pm 1.47$  meq/kg. Only one sample exceeded the FSSAI acceptable limit of 10 meq/kg (Figure 1). Peroxide values during frying were monitored. In two samples, PV increased from  $1.5 \pm 0.19$  to  $5.1 \pm 0.57$  meq/kg and from  $2.9 \pm 0.37$  to  $4.0 \pm 0.49$  meq/kg at mid-frying, then decreased to  $0.07 \pm 0.01$  and  $1.6 \pm 0.09$  meq/kg, respectively, at the end of frying. Similar results were reported by Ahmed Ali Massad et al. (2021) in oil used for frying chicken.

## Microbial quality assessment

The microbial quality of ten shawarma samples was assessed by total plate count (TPC) and yeast and mold count (YMC) methods. All samples exceeded the FSSAI acceptable limit of  $1 \times 10^3$  CFU/g for both methods. The TPC ranged from  $5 \times 10^3$  to  $8.5 \times 10^4$  CFU/g, while YMC ranged from  $1.5 \times 10^2$  to  $1.2 \times 10^4$  CFU/g. Similarly, Odu and Akano (2012) reported total viable counts ranging from  $2.0 \times 10^3$  to  $1.6 \times 10^6$

CFU/g, and Salem et al. (2016) observed yeast and mold counts ranging from  $9 \times 10^1$  to  $1.9 \times 10^2$  CFU/g.

## Identification and characterization of *Salmonella*, *E. coli* and *Listeria*

*Salmonella* isolates were identified by red colonies with black centers on XLD agar and light purple colonies with a purple halo on *Salmonella* chromogenic agar plates (Figure 2). Among the ten samples tested, two samples showed confirmed growth of *Salmonella* colonies. The isolates were further confirmed by twelve biochemical tests (Table 1) and PCR targeting a 284 bp fragment of the *Salmonella*-specific *invA* gene (Figure 3). Similarly, *Salmonella* spp. have been isolated from various food samples, including ready-to-eat foods, as reported by Razaa et al. (2021), Ashrafudoulla et al. (2021), Alharbi et al. (2019), Aktar et al. (2016), Akbar and Anal (2015) and El-Shenawy et al. (2011) and characterized using standard biochemical tests. Tarabees et al. (2017) identified and characterized *Salmonella* isolates by multiplex PCR using 17 virulence genes, including the *invA* gene.

*E. coli* was identified by yellow colonies on XLD agar and blue colonies on *Salmonella* chromogenic agar plates (Figure 2). Among ten samples tested for the presence of *E. coli*, six samples showed confirmed growth of colonies. Further confirmation of positive *E. coli* colonies was performed using twelve biochemical tests (Table 1). Similar studies have been reported by Ema et al. (2022), Giri et al. (2021), Yang et al. (2020), Ahmad et al. (2013), and Biswas et

*al.* (2010). For molecular confirmation, an 884 bp fragment of the *E. coli*-specific *uspA* gene was amplified by PCR (Figure 3).

*Listeria* was identified by blackening of the medium around colonies on Oxford agar plates (Figure 2). Six out of ten samples tested showed confirmed growth of *Listeria* colonies. *Listeria* spp. have been isolated from contaminated food samples, including milk, meat, poultry, seafood, dairy products and plant-derived products, as reported by Fatima *et al.* (2024), Goudar *et al.* (2021), El-Shenawy *et al.* (2011) and Cocolin *et al.* (2002). Further confirmation of the isolates was performed using twelve biochemical tests (Table 1) and PCR targeting a 457 bp fragment of the *Listeria*-specific *iap* gene (Figure 3). Similarly, Al-Brefkani *et al.* (2019), Nayak *et al.* (2015), and El-Shenawy *et al.* (2011) identified *Listeria* spp. from milk, milk products, meat and fish samples using *iap* gene amplification.

### **Estimation of Histamine content by HPTLC**

Among ten shawarma samples tested for histamine by HPTLC, three samples showed positive results, with concentrations ranging from 17 to 70 ppm (Figure 4). However, all values were below the FAO/WHO maximum safe level of 200 ppm. Sallam *et al.* (2021) also detected histamine in beef and chicken shawarma.

### **CONCLUSION**

Shawarma is consumed by a diverse spectrum of the population worldwide. Chicken is the major ingredient in shawarma

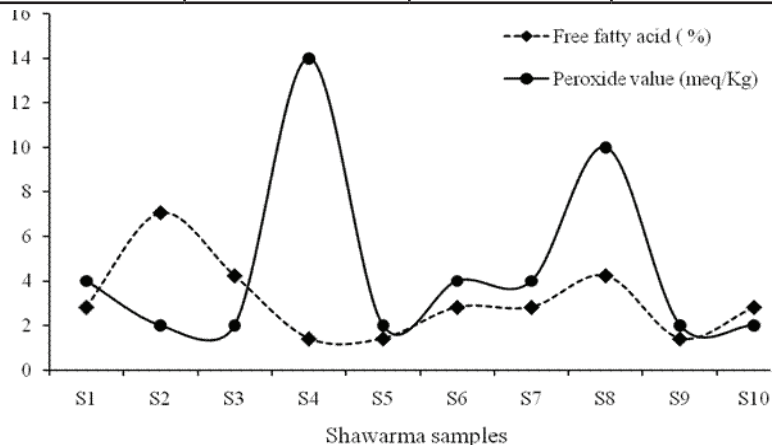
preparation; however, it is a potential source of microbial contamination if not properly cooked. The present study revealed that ready-to-eat chicken shawarma sold in Chennai, Tamil Nadu, was contaminated with pathogenic bacteria, including *Salmonella*, *E. coli*, *Listeria*, yeast, and mold, as confirmed by both biochemical tests and PCR. Histamine was also detected in some samples, which may pose a risk of allergic reactions to consumers. The high microbial load in certain contaminated shawarma samples exceeded regulatory limits, predisposing them to food spoilage. To prevent pathogenic contamination, vendors should follow safety protocols by adhering to proper cooking procedures, using high-quality chicken, and maintaining hygienic handling practices to provide safe and nutritious food to the community. Additionally, labeling shawarma products may help reduce the risk of allergic reactions and ensure adherence to food safety standards.

### **ACKNOWLEDGEMENT**

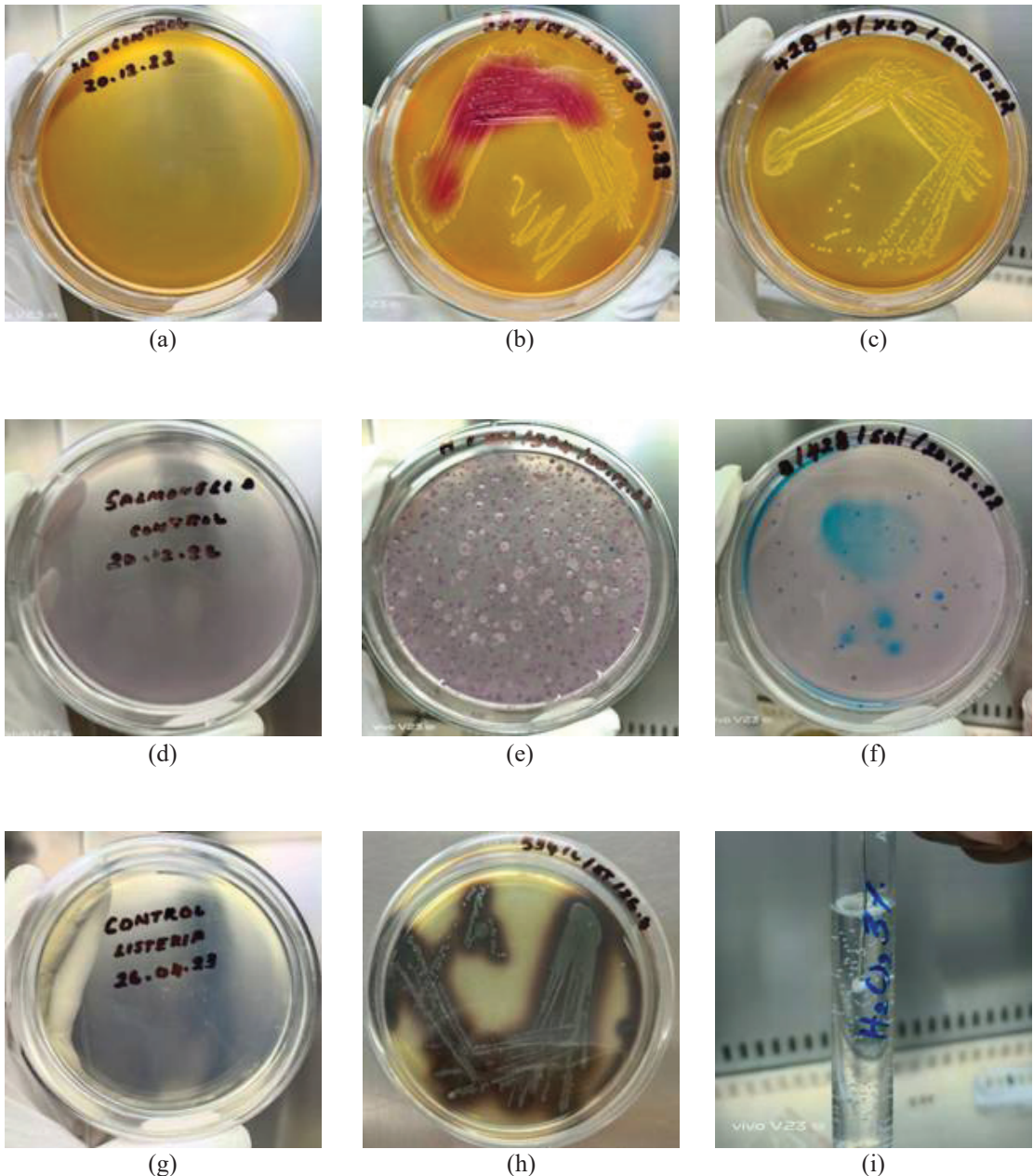
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**Table.1. Biochemical characteristics of Salmonella, E. coli and Listeria culture**

S. No.	Test	<i>Salmonella</i>	<i>E. coli</i>	<i>Listeria</i>
1	Methyl Red	Red	Red	Red
2	Voges Proskauer's	Yellow	Pinkish red	Red
3	Urease	Orangish yellow	-	-
4	H2S Production	Orangish yellow	-	-
5	Citrate utilization	Green	Blue	-
6	Lysine utilization	Purple	Purple	-
7	ONPG	Colourless	Yellow	-
8	Lactose	Yellow	Yellow	Yellow
9	Arabinose	Yellow	-	-
10	Maltose	Yellow	-	-
11	Sorbitol	Yellow	Yellow	-
12	Dulcitol	Yellow	-	-
13	Indole	-	Reddish pink	-
14	Glucuronidase	-	Bluish green	-
15	Nitrate reduction	-	Pinkish red	Colourless
16	Glucose	-	Yellow	Yellow
17	Sucrose	-	Yellow	Yellow
18	Catalase	-	-	Effervescence from the loop
19	Esculin hydrolysis	-	-	Dark
20	Xylose	-	-	Yellow
21	a-methyl-dmannoside	-	-	Red
22	Rhamnose	-	-	Red
23	Manitol	-	-	Yellow



**Fig.1. Rancidity profile of ten shawarma samples. Dotted line indicates free fatty acid content in percent and solid line indicates peroxide value in meq/kg**



**Fig.2. Identification of *Salmonella*, *E. coli* and *Listeria* cultures in growth medium. (a) XLD agar control plate; (b) Red with black centered *Salmonella* colonies on XLD agar plate; (c) Yellow coloured *E. coli* colonies on XLD agar plate; (d) *Salmonella* chromogenic agar control plate; (e) Light purple coloured with a purple halo *Salmonella* colonies on *Salmonella* chromogenic agar plates; (f) Blue coloured *E. coli* colonies on *Salmonella* chromogenic agar plates; (g) Oxford agar control plate; (h) Blackening of medium around the *Listeria* colonies on Oxford agar plate; (i) Effervescence coming out from the loopful of *Listeria* culture.**

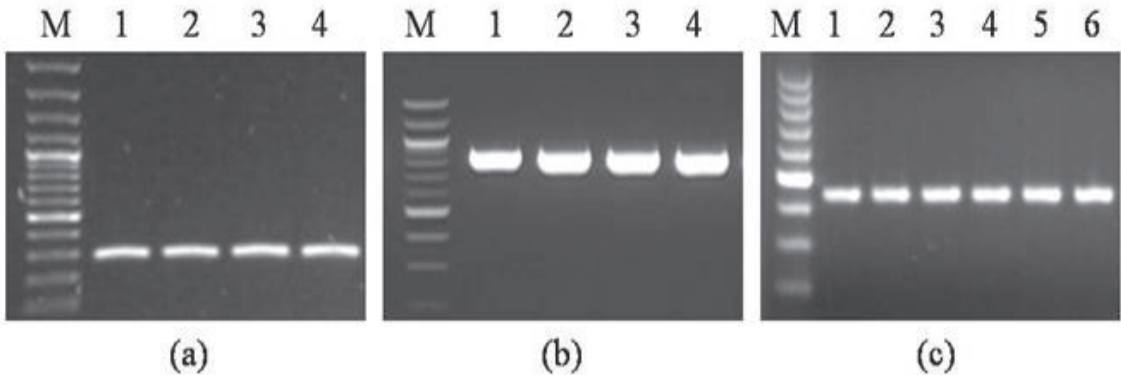
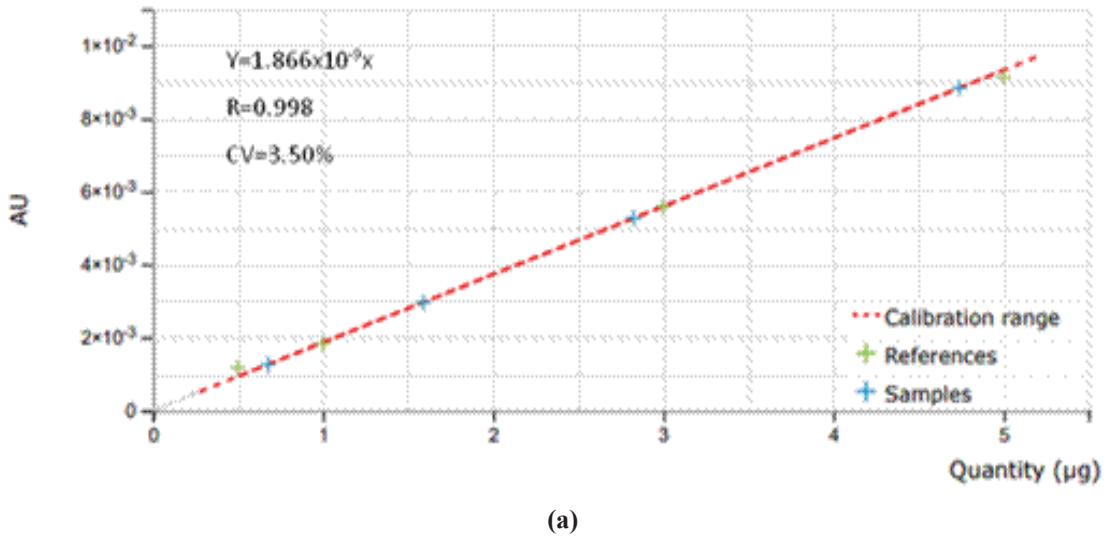
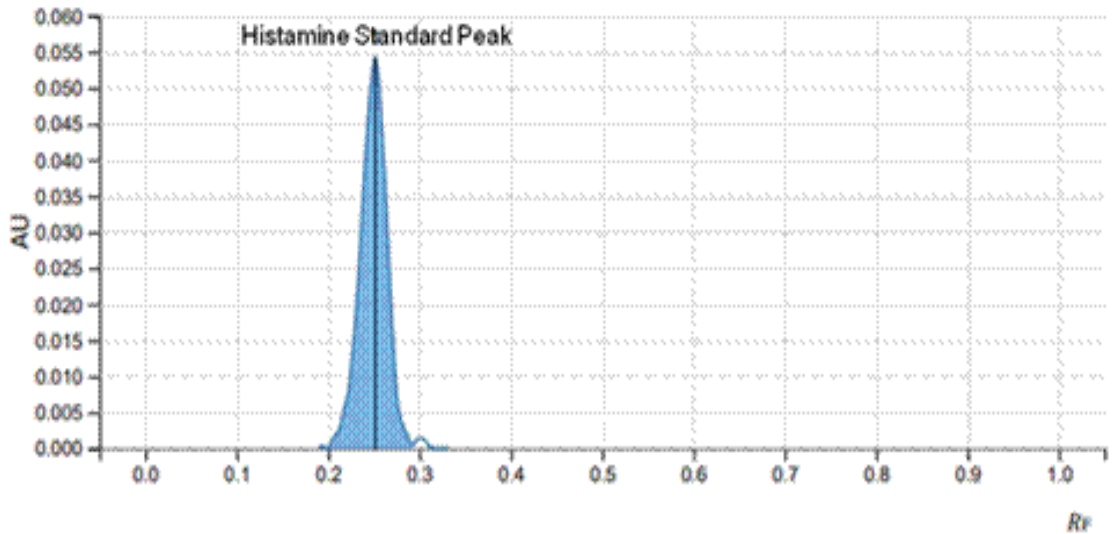


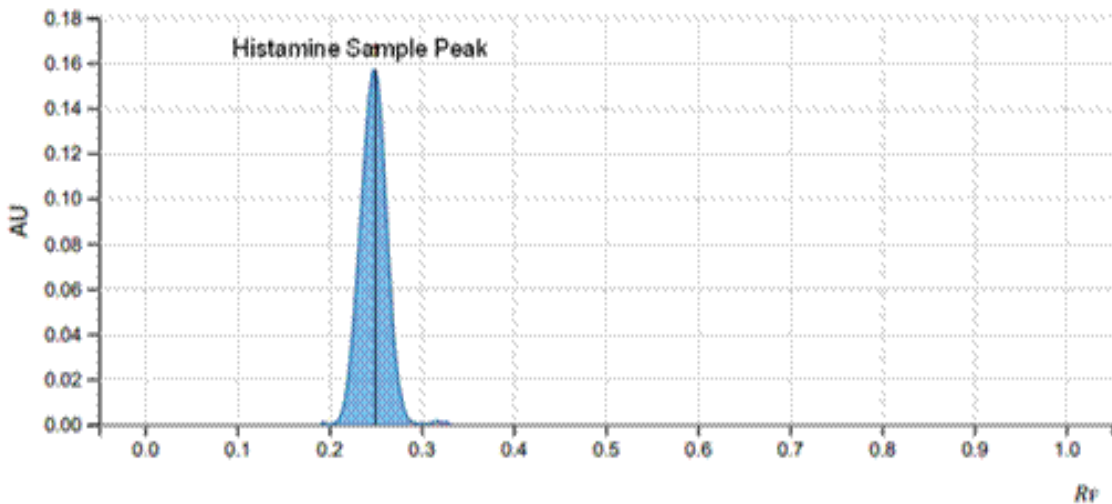
Fig.3. Agarose gel electrophoresis of PCR products amplified from *Salmonella*, *E.coli* and *Listeria* isolates. (a) 284 bp amplicon of *invA* gene amplified from *Salmonella* isolates. M – DNA ladder, Lane 1 to 4 – *Salmonella* isolates; (b) 884 bp amplicon of *uspA* gene amplified from *E.coli* isolates. M – DNA ladder, Lane 1 to 4 – *E.coli* isolates; (c) 457 bp amplicon of *iap* gene amplified from *Listeria* isolates. M – DNA ladder, Lane 1 to 6 – *Listeria* isolates.

Area calibration for substance Histamine @ 410 nm:





(b)



(c)

Fig.4. HPTLC analysis of histamine in shawarma samples at 410 nm with R<sub>f</sub> 0.259. (a) Calibration curve (linearity) of histamine standard with correlation coefficient (R) 0.998 and coefficient of variation (CV) 3.50%; (b) Densitometric chromatogram of histamine from standard; (c) Densitometric chromatogram of histamine from sample.

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