SPOILAGE BACTERIA IN FRESH BEEF AND THEIR PRESERVATION BY ORGANIC ACIDS

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Abstract: Outbreaks of food borne spoilage continue to draw public attention to food safety. The objective of the study was to assess the bacteriological quality of fresh raw beef sold in three different (Chidambaram, Bhuvanagiri and Parangipettai) markets of Cuddalore district. Among the three market Chidambaram (4.28×10^4) market contain high mean bacterial counts. Now a day several reports have demonstrated the efficacy of using chemical to control the growth of food spoilage. In the present study the three organic acid viz., acetic acid, citric acid and lactic acid was used to test the beef isolated bacteria by well diffusion method. Citric acid found to be the effective inhibitor (20.25mm) compare with acetic acid and lactic acids.

Key words: Beef, Spoilage bacteria, bacteriological quality, Organic acids, Well diffusion method.

I. INTRODUCTION:

Meat is highly valued food product for human consumption because it is a good source of all essential amino acid and a major source of B – complex vitamins and minerals. Its distinctive flavour makes it one of the most preferred foods. Meat has been described as the most perishable of all important foods since its contains sufficient nutrient needed to support the growth of microorganisms .The chemical composition of meat is approximately water 71-76%, protein 20-22%, lipid 3-8%, carbohydrate 1.2%, soluble and non proteins substances nearly 2.3% provides an ideal substrate for microbial growth (Samelis, 2006). Meat is considered as the most nutritive source of protein consumed by humans. Most meat have high water content corresponding to the water activity approximately 0.98 which is most suitable for microbial growth (William C Frazier *et al.*, 2014).

Meat is subjected to changes by its own enzyme, by microbial action and its fat may be oxidized chemically microorganisms grow on meat causing visual, textural and organoleptic change when they release metabolites (Ukut *et al.*, 2010). In living animals, those surfaces communicating with environment, harbour variety of microorganisms. When tissues are removed from the carcass and exposed to the environment, the sterile surfaces will become contaminated with microorganisms. The contaminating organisms are derived mainly from the hide of the animal and also comprise organisms that originate from both faeces and soil. In addition, processed meat foods are more prone to contamination with pathogenic microorganisms during the various stages of processing. The most important pathogens associated with meat include *Salmonella, Staphylococcus, Escherichia Coli, Clostridium, Campylobacter, Listeria, Bacillus, Vibrio* (Aastha Acharya *et al.,* 2016).

The major challenge for the food industry is to eliminate these undesirable changes that occur in foods as a result of the action of microorganisms and ensuring maximum security in foods. Chemical additives like organic acids which include lactic, acetic and citric acid have been used to combat the action of theses microorganisms (Uzoh *et al.,* 2016). There are a large number of chemicals that can serve as food preservatives but a small number is allowed in foods due to food and drug administration (FDA) rules which should be

strictly adhered to. Hence some of these compounds that exhibit antimicrobial effect in vitro do not show that when added to foods. In order to enhance the shelf life of foods, chemical preservatives have been used.

In lieu of the above justification, the present endeavour was to assess the bacteriological quality of raw beef and evaluate the antibacterial activity of three chemical food preservatives against beef associated bacterial isolates.

II. MATERIAL AND METHODS

2.1 Sample collection:

Freshly slaughter beef sample were collected in sterile polythene bag from three different places (Chidambaram, Bhuvanagiri and Parangipettai market) of Cuddalore district. Collect three samples in each market.

2.2 Sample preparation:

Ten grams (10g) of each beef sample weighed out and homogenized into 90ml of buffered peptone water (Ukut *et al*., 2010) using a sterile blender.

2.3 Total viable count (TVC) of bacteria:

one ml of sample was serially diluted up to 10^{-6}. Then 1ml of sample from 10^{-4} to 10^{-6} was inoculated into plated Nutrient agar medium incubation for 24hrs at 37°C. The TVC of each plate were expressed as colony forming unit of the suspension (cfu/ml).

2.4 Isolation of pathogens

2.4.1 For *Samonella* spp and *Shigella* spp

Two ml of beef sample rinsate was transferred to Double strength lactose broth incubated for 24hrs at 37°C. A loopful of lactose broth was streaked on Salmonella – Shigella (SS) Agar medium. Gram's staining was done and colony characteristics were noted.

2.4.2 For *Staphylococcus* spp

Two ml of beef sample rinsate was transferred to nutrient broth incubated for 24hrs at 37°C. A loopful of Nutrient broth was streaked on Mannitol Salt Agar (MSA) medium. Gram's staining was done and colony characteristics were noted.

2.4.3 For *vibrio* spp

Two ml of beef sample rinsate was transferred to alkaline peptone water incubated for 24hrs at 37°C. A loopful of alkaline peptone water was streaked on Thiosulphate Citrate Bile Salt (TCBS) Agar medium. Gram's staining was done and colony characteristics were noted.

2.4.4 For *E.coli*

Two ml of beef sample rinsate was transferred to MacConkdy broth incubated for 24hrs at 37°C. A loopful of MacConkdy broth was streaked on Eosin Methlene Blue (EMB) Agar medium. Gram's staining was done and colony characteristics were noted.

2.5 Biochemical characterisation:

Biochemical characterisation of the bacteria was done by performing specific test such as Indole, Methyl red, Voges Proskauer ,Citrate test, Catalase, Oxidase, Triple Sugar Iron (TSI), Starch hydrolysis, Urease test, Nitrate reduction test.

2.6 Evaluation of chemical food preservatives for their antibacterial activity

2.6.1 Preparation of stock solution

Different concentration (200,400,600,800,100 µg/ml) of acetic acid, citric acid and lactic acid were prepared (Elamathy *et al*., 2013).

2.6.2 Antibacterial activity by agar well diffusion method:

Plate Count Agar (PCA) plates were inoculated with 100µl of beef associated bacterium and spread with sterile swab. Wells of 8mm size were made with sterile cork borer into agar plates containing the bacterial inoculums and the lower portion sealed with a little molten agar medium. 100µl of chemical preservatives was poured into a well of inoculated plates. Sterilized distilled water was used as a control which was introduced into a well instead of chemical preservatives. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extract into the agar. After incubation for 24hrs at 37°C, the plates were observed. If antibacterial activity was present on the plates, it was indicated by an inhibition zone surrounding the cell containing the chemical preservatives. The zone of inhibition was measure with antibiotic zone scale in millimetres (Ram kumar pundir and Pranay jain, 2011)

III. RESULT AND DISCUSSION

3.1 Total Viable Count (TVC):

Table 3.1: Area basis total viable bacterial count from collected fresh beef.

Sample	Samples	Mean Total viable bacterial count (CFU/g)	Mean total bacterial count (CFU/g) from individual market
Chidambaram	A1	4.84×10^4	4.28×10^4
	B1	4.28×10^4	
	C1	3.74×10^4	
Bhuvanagiri	A2	3.18×10^4	3.42×10^4
	B2	4.12×10^4	
	C2	2.98×10^4	
Parangipettai	A3	3.45×10^4	3.74×10^4
	B3	2.98×10^4	
	C3	4.80×10^4	

*Three replication per sample

The mean microbial load of fresh beef from Chidambaram market was 4.28×10^4 and in Bhuvanagiri market was 3.42×10^4 and in Parangipettai market was 3.74×10^4 as shown in table 3.1.

In that three samples Chidambaram market sample was high microbial count (4.28×10^4), compare with Bhuvanagiri and Parangipettai. samples. The minimum count occurred in Bhuvanagiri (3.42×10^4) sample. Collected three samples had the potential incidence of bacteria for spoilage the fresh meat.

3.2 Isolation and identification:

Table 3.2: Morphological and Biochemical characteristics of isolated organisms

Parameters	Isolates			
	E.coli	Salmonella typhimurium	Staphylococcus aureus	Vibrio parahaemolyticus
Gram's staining	-	-	+	-
Cellular morphology	Rod	Rod	Cocci	Curved rod
Nutrient agar	Circular and colourless colonies	Smooth, slimy, Colourless colonies	Smooth, golden yellow colonies	Large, smooth,Colourless colony
Selective medium	EMB	SS Agar	MSA	TCBA
Colony character	Green metallic sheen.	Black colour colonies	Golden yellow colonies	Green yellow colonies.
Indole	+	-	-	+
Methyl red	+	+	+	+
Voges Proskauer	-	-	+	-
Citrate test	-	+	+	-
Catalase	+	+	+	+
Oxidase	-	+	-	+
TSI	Slant- Red Butt- yellow	Butt- Black	N/A	Slant- Red Butt – Yellow
Starch hydrolysis	-	-	-	
Urease test	-	-	-	-
Nitrate reduction test	-	+	-	+

*N/A = Not applicable, (+) = positive result, (-) = Negative result

Table 3.2 shows that the characteristic growth of microorganisms in differential media and various biochemical tests indicated them as E.coli, Salmonella typhimurium, Staphylococcus aureus, vibrio parahaemolyticus (Farhana jahan *et al.*, 2015)

3.3 Preservation by organic acids:

Table 3.3: Antibacterial activity of Organic acids against beef isolated bacteria

Organic acids	Organisms	Concentration of organic acids (µg/ml)						
		200	400	600	800	1000	Mean	Average inhibition of individual chemical (mm)
		Zone of inhibition (in mm)						
Acetic acid	*E. coli*	11.34	13.66	18.12	20.00	23.60	17.22	19.13
	Salmonella typhimurium	13.33	16.65	20.60	23.80	26.59	20.19	

	Staphylococcus aureus	12.56	14.23	18.66	22.00	26.82	18.85	
	Vibrio parahaemolyticus	15.00	16.22	20.00	24.00	26.02	20.25	
Citric acid	*E. coli*	12.20	15.00	19.82	22.66	25.00	18.94	20.25
	Salmonella typhimurium	14.00	18.33	21.00	24.45	26.69	20.89	
	Staphylococcus aureus	15.66	17.10	19.60	22.00	26.00	20.07	
	Vibrio parahaemolyticus	14.32	18.90	20.21	24.90	27.10	21.09	
Lactic acid	*E. coli*	11.33	13.00	14.33	18.00	22.10	15.75	18.09
	Salmonella typhimurium	12.66	15.06	18.33	23.00	25.32	18.87	
	Staphylococcus aureus	11.33	15.00	17.00	20.33	23.66	17.46	
	Vibrio parahaemolyticus	15.34	17.54	20.00	23.21	25.20	20.26	

*Average of three replications.

Table 3.3 show that citric acid recorded a highest mean inhibition zone of 20.25 mm followed by acetic acid (19.13 mm) and lactic acid (18.09 mm). Uzoh *et al.,* 2016, Study revealed that citric acid recorded highest inhibition compare with acetic and citric acid.

SUMMARY:

The present study reveals the fact that raw meat from retail outlets is heavily contaminated with the high incidence of bacterial pathogens. So chemical preservation is one of the method to minimize the bacterial load. It is imperative that basic hygienic practices to be incorporated in retail outlets to ensure food safety. Training should be given to meat handlers and butchers regarding food safety practices and proper inspection procedures should be strictly adhered to minimise the contamination of raw meat sold in market places.

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