International Journal of Advanced Multidisciplinary Research (IJAMR)
ISSN: 2393-8870
www.ijarm.com

Research Article
Antimicrobial effect of *Camellia sinensis* on chicken meat.

[1]Karandikar Pranali, [2]Bhatte Sukhada and [3]Ghayal Madhura.

[1]Student, Department of Food, Nutrition and Dietetics, College of Home Science, Nirmala Niketan, 49, New Marine Lines, Mumbai 400 020, India
[2]Lecturer, Department of Food, Nutrition and Dietetics, College of Home Science, Nirmala Niketan, 49, New Marine Lines, Mumbai 400 020, India
[3]Associate Professor of Microbiology, Department of Microbiology, Jai Hind College, Mumbai 400020
Corresponding Author : *pranali301@gmail.com*

Keywords

Camellia sinensis,
Green tea,
Chicken,
Antimicrobial agents,
Meat preservation

Abstract

Camellia sinensis (Tea) is one of the most popular nonalcoholic beverages, consumed by over two-thirds of the world's population. The aim of the present study was to establish the antimicrobial effect of green tea on spoilage organisms from chicken. The spoilage organisms from chicken were isolated and the probable spoilage organisms were characterized as *S. aureus, E.coli, Bacillus* spp and *Salmonella typhi.* The antimicrobial activity of three different specimens of green tea samples viz. Lipton tea (commercial processed tea), Organic India green tea (organic tea) and Tenfu tea (Chinese tea) was checked against the standard. The antimicrobial activity was tested against the tea samples at varied boiling times and no significant difference in the activity of green tea across the boiling time was found ($p > 0.05$). The Lipton tea was found to be most inhibitory towards the organisms and the least inhibitory was Organic India Green tea; their Mean zone of inhibition being 9.5 ± 2.08 mm and 2.25±4.5 mm respectively at 5mg/ml. The Minimum Inhibitory Concentration (MIC) for Lipton tea and Tenfu tea was 2.5 mg/ml for *E.coli.* Lower strength of the tea samples were not bacteriocidal. However, bacteriostatic effect was confirmed by higher strengths of all the tea samples. It can be thus concluded that green tea has antimicrobial activity against spoilage organisms from chicken and can be effectively used as a preservative agent to increase the shelf life of chicken.

Introduction

Spoilage of food occurs by three ways i.e. by chemical (chemical reactions between two or more food components), biological (microbial contamination) or physical (change in texture and appearance). The most common perishable foods to be spoiled are the foods that have high water activity like fruit juices and those that have high amounts of nutrients like milk and milk products, animal products and cereals (Gustavsson, Cederberg and Sonesson, 2011). Poultry meat being a nutrient dense food product is therefore highly susceptible to spoilage from microbes like *Bacillus subtilis* which can metabolize proteins with the help of protease enzyme and convert them to polypeptides and further amino acids (Khan et al., 2011). The common spoilage organisms related to poultry are *Brucella, Mycobacterium tuberculosis, Coxiella, Listeria, Campylobacter,* beta-hemolytic *Streptococococci,* Enteropathogenic *E.coli, Staphylococci* and *Salmonella, parasites* and viruses (Frazier and Westhoff, 1999).

A variety of physical preservation techniques like low temperature storage, freezing, radiation and modified atmosphere packaging (Zhou, Xu and Liu, 2010) as well as chemical preservation techniques like using alcohols, phenols and nitrates and nitrites are used to preserve poultry meat (Jay, 2000). The drawback of these methods is that they are costly and could be inefficient to inhibit the microbes completely; hence, antibiotics/ chemical antimicrobials are used for preventing the spoilage. The overuse of antibiotics has led to the rise of antibiotic resistant strains. For example S*taphylococci* which cause Staphylococcal food poisoning have become antibiotic resistant to Vancomycin antibiotic (Dalcin et al., 2013). Daglia (2012), considers an emerging urgent need for a new antimicrobial agent as very urgent due antibiotic resistance shown by major organisms.

Tea is one of the most popular nonalcoholic beverages, consumed by over two-thirds of the world's population

because of its refreshing, mild stimulant and medicinal properties. Green tea polyphenols like catechins have shown some protection against the *Streptococcus mutans* which cause dental infections in humans, improved gut bacteria and also helps to reduce the microbial load during cancers like reduction in population of *H.pylori* during gastric cancer (Afaq et al., 2004). Also, antimicrobial effects of green tea catechins can help in protection from microbial load during vaginitis (Kim et al., 2013) along with anti-aflatoxin activity on *Aspergillus flavus*. Also, Gong (2006), described the use of tea polyphenols on the antibiotic resistant *S.aureus*.

The present study aims at studying the efficacy of various strengths of green tea samples as an antimicrobial agent in preservation of raw chicken samples.

Materials and Methods

Isolation and identification of spoilage organisms

The chicken samples were subjected to spoilage by keeping raw chicken samples in open Petri plates. The swabs were taken at a regular interval of 15 mins from the chicken samples and its turbidity was checked at 660 nm on a spectrophotometer. The increase in the intensity of the turbidity over the period of time indicated the increase in the microbial cell load. A graph of absorbance against time was plotted to check the time at which there was maximum spoilage in the chicken sample.

Once the peak spoilage time was known, swabs from the sample were taken at that particular time. The swabs so taken were plated on selective media Mac Conkeys agar, Salt Mannitol agar and Salmonella Shigella agar and non-selective media (Nutrient agar). Post incubation, the colony characteristics and differential staining technique (Gram Staining) was done for identification of the spoilage organisms.

Antimicrobial effect of Green tea samples

Sample Preparation

5 grams of three green tea samples (Lipton green tea, Organic India and Tenfu tea) were taken in 100 ml of distilled water. Each of the samples was boiled for 5 minutes and 10 minutes separately. The concentration of the stock solution was thus 5 mg/ml.

Paper Diffusion Assay

The laboratory cultures of the spoilage organisms which were identified in the previous step were used to assess the antimicrobial effect of the above mentioned green tea samples by the paper diffusion method. The spoilage organisms were streaked on Nutrient agar plates. Sterile discs of Whatmann filter papers were dipped in the tea samples and then placed on the swabbed plate. After incubation for 24 hours at 37 °C, the zone of inhibition for the tea samples were checked.

Estimation of Minimum Inhibitory Concentration of green tea (MIC)

The stock sample used for assessment of zone of inhibition of green tea was diluted to 1:2, 1:4, 1:8 and 1: 16 strengths and checked for its antimicrobial activity at all dilutions on the same laboratory cultures. 5 sterile tubes of 3 ml nutrient broth were taken. To the 1st tube 3 ml of green tea sample is added and was mixed well. 3 ml from the 1st tube is transferred to the 2nd tube. The dilutions are continued till the 4th tube and 3 ml from the 4th tube is discarded. The 5th tube was the positive control. A separate tube for sample control was also prepared. Similar sets of tubes were made for all the test microbes. The tubes were incubated at 37 °C for 24 hours and the turbidity of the samples was checked. The highest dilution without growth is the minimum inhibitory concentration.

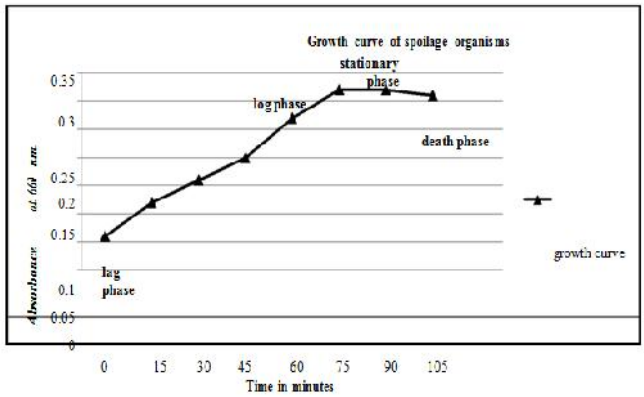
Estimation of Minimum Bacteriocidal concentration (MBC Assay)

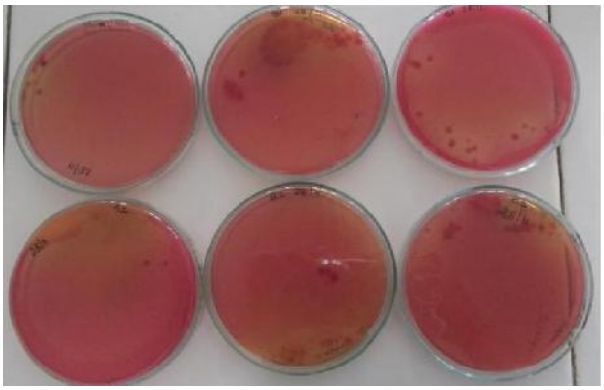
The green tea when added to the nutrient broth gave a turbid appearance to the sterile nutrient broth tubes. Thus, to check if the turbidity of the samples was due to microbial growth or tea samples the samples were then plated on nutrient agar plates and again incubated for 24 hours at 37 °C. The dilution at which there was no growth was termed as bactericidal concentration and the concentration at which there is steady growth was termed as bacteriostatic concentration.

Results

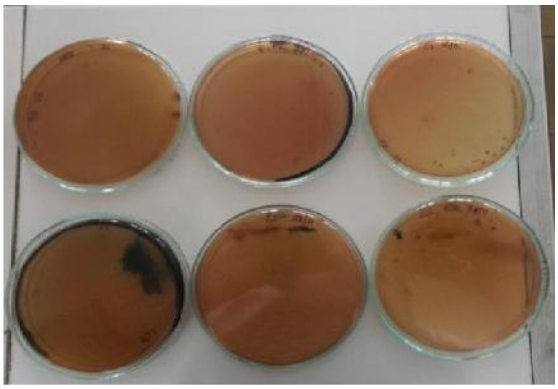
Isolation and identification of spoilage organisms

The peak growth was seen when the chicken is kept for 75 minutes and later it entered the stationary phase. With the help of the graph, the peak growth was determined to be from 80 minutes of spoilage until 90 minutes of spoilage.

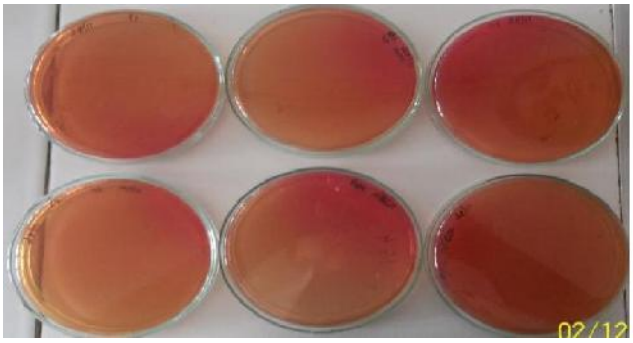




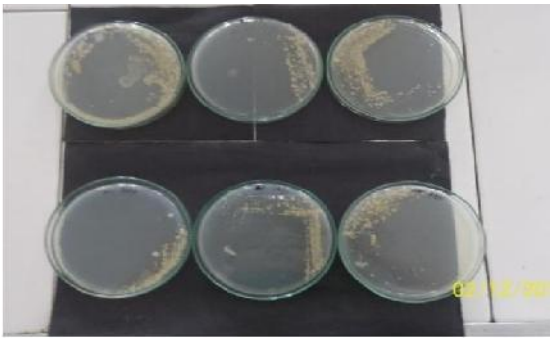
(a) Mac Conkeys Agar



(c)Salmonella Shigella agar



(b) Salt Mannitol Agar



(d) Nutrient agar

The spoilage organism thus identified were gram negative colonies of *E.coli* on Mac Conkeys agar and *Salmonella spp* on Salmonella Shigella agar and gram positive organisms like *Staphylococcus aureus* on Salt mannitol agar and *Bacillus spp* on Nutrient agar. .

Paper Diffusion Assay

The green tea samples viz., Lipton green tea, Organic India green tea and Tenfu tea were labeled as sample A, B and C respectively. The antimicrobial sensitivity for the standard laboratory cultures of *S.aureus, E.coli, S.typhi and Bacillus spp* was checked using paper diffusion assay. The concentration of the tea sample taken was 5 mg/ml. Amongst the three green tea samples, Sample A showed greatest inhibition against the standard cultures.

	Tea samples	Sample A (5mg/ml)		Sample B (5 mg/ml)		Sample C (5mg/ml)	
Cultures	Boiling time (minutes)	5'	10'	5'	10'	5'	10'
		Zone of inhibition (mm)					
E.coli		7	6	0	0	8	8
Bacillus		10	9	9	0	9	10
S.aureus		9	10	0	0	6	8
S. typhoid		12	13	0	0	10	7
	Mean ± S.D.	9.5 ± 2.08	9.5 ± 2.88	2.25 ± 4.5	0 ± 0	8.2 ± 1.2	8.2 ± 1.2

Tea sample	**Sample A**				**Sample B**				**Sample C**			
Dilutions	**1:2**	**1:4**	**1:8**	**1:16**	**1:2**	**1:4**	**1:8**	**1:16**	**1:2**	**1:4**	**1:8**	**1:16**
Concentrations(mg/ml)	**2.5**	**1.25**	**0.625**	**0.312**	**2.5**	**1.25**	**0.625**	**0.312**	**2.5**	**1.25**	**0.625**	**0.312**
Cultures												
E.coli	-	+	+	+	NA	NA	NA	NA	-	+	+	+
S.aureus	+	+	+	+	NA	NA	NA	NA	+	+	+	+
S.typhi	+	+	+	+	NA	NA	NA	NA	+	+	+	+
Bacillus spp	+	+	+	+	+	+	+	+	+	+	+	+

Key: +ve: growth present -ve: growth absent

NA: test was not performed

Discussion

The peak spoilage time for chicken meat samples is after 75 minutes of exposure to air. The major spoilage organisms for chicken meat are *S.aureus, E.coli, Salmonella* and *Bacillus*. Green tea shows antimicrobial effect on all the organisms at varied boiling times of 5 minutes and 10 minutes. There is no significant difference between the two boiling times ($t=1$; $p>0.05$, df 6). Green tea has bacteriostatic effect even at 0. 312 mg/ml concentration for all the samples. Sample A and C are bactericidal at 2.5 mg/ml concentrations for *E.coli* and bacteriostatic for all the other organisms on further dilutions.

Conclusion

The aim of the present study was to determine the antimicrobial effect of green tea on spoilage organisms in chicken at different boiling times. Green tea has no significant difference in its antimicrobial activity at different boiling times. Also, Green tea showed significant antimicrobial activity against all the spoilage organisms isolated from chicken samples at concentrations as low as 0.312 mg/ml. Thus, it can be concluded that green tea is an effective antimicrobial agent for the control of spoilage in chicken meat.

References

1. G.H. Zhou, Xu and Liu X.L. Xu, Y. Liu (2010) Preservation technologies for fresh meat –A review in *Meat Science* 86 (1).
2. James.M.Jay (2000), *Modern food microbiology* 2nd edition page no 330- 345 : –Aspen publication
3. Jenny Gustavsson Christel Cederberg Ulf Sonesson, (2011). global food losses and food waste Swedish Institute for Food and Biotechnology (SIK)
4. Maria Daglia (2012) Polyphenols as antimicrobial agents -*Current Opinion in Biotechnology*
5. Mohsin Ahmad Khan1, Nadeem Ahmad1, Ahmad Usman Zafar2, Idrees Ahmad Nasir1 and Muhammad Abdul Qadir, (2011) - Isolation and screening of alkaline protease producing bacteria and physio-chemical characterization of the enzyme - *African Journal of Biotechnology* Vol. 10(33), pp. 6203-6212
6. Paula Dalcin Martins, Taiana Trindade de Almeida, Ana Paula Basso, Tiane Martin de Moura, Jeverson Frazzon, Eduardo César Tondo, and Ana Paula Guedes Frazzon-September (2013) -Coagulase-Positive Staphylococci Isolated from Chicken Meat: Pathogenic Potential and Vancomycin Resistance - *Foodborne Pathogens and Disease* 10(9) 771-776

7. Weiduo Si, Joshua Gong, Rong Tsao, Milosh Kalab, Raymond Yang, Yulong Yi, (2006) Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract in *Journal of Chromatography A* 1125(2),
8. William Carol Frazier and Westhoff, 1999, *Food microbiology*, McGraw Hill Publications.
9. Yong-wan Kim (2013). Antimicrobial and antifungal effects of green tea extracts against microorganisms causing vaginitis –in *Food Science and Biotechnology* 22,(3,) 713-719