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Research Article

ASSESSMENT OF MICROBIAL QUALITY OF FRESH STRAWBERRIES IN CONSUMER MARKETS OF COIMBATORE CITY, TAMIL NADU, INDIA

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Abstract- The fresh strawberries are highly prone to contamination by food borne pathogens and so this study was conducted to analyze the microbial quality parameters such as total aerobic mesophilic microbial count, fungal count, Enterobacteriaceae colony count, total coliforms count, fecal coliforms count, *E. coli* count and presence of *Salmonella* in fresh strawberries sold in supermarkets around the areas of west Coimbatore city. In addition, the two methods for coliforms detection namely Most Probable Number (MPN) and petrifilm methods were compared. The results showed that the total aerobic mesophilic count ranged from 5.53 to 6.61 log cfu/g and the fungal count ranged from 1.42 to 4.46 log cfu/g. The Enterobacteriaceae colony count ranged from 1.42 to 3.81 log cfu/g with supermarket 4 having the highest count. On comparing the two methods for coliforms detection, it was found that the petrifilm count was within the corresponding MPN range at 95% confidence limit for all the analysed samples. The total coliforms count showed huge difference among the strawberry samples in both MPN and petrifilm methods. Market 1 had the highest coliform count in both methods. The fecal coliforms were found in samples from supermarkets 1, 3 and 5. There was no *E. coli* and *Salmonella* in the fresh strawberry samples but the presence of fecal coliforms in some samples indicate the possibility of fecal contamination which reflects the need for Good Agricultural Practices (GAP) in primary production and Good Hygiene Practices (GHP) in pre-harvest and post-harvest operations.

Keywords- *Strawberry, Microbial quality, Coliforms, Food safety*

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Introduction

Fresh fruits and vegetables are the important components of healthy diet which are having increasing demand all around the world. Despite the fact of high health benefits, there is high concern on the food safety associated with consumption of the fresh fruits and vegetables [1]. Strawberries (*Fragaria* X *ananassa*) are one of the important functional food's rich in anti-oxidants, folic acid and other beneficial phytochemicals. They are often consumed fresh and they have very short shelf life due to their soft nature and high sensitivity to senescence and fungal spoilage. Strawberries are picked manually and packed in punnet boxes without any disinfection step as it hastens the deterioration process [2]. In spite of the inherent acidity of strawberries, the avoidance of decontamination process allows the food-borne pathogens to survive on the surface of fresh strawberries which pose a major threat to food safety [3]. In 2011, *E. coli* O157 outbreak was reported in Oregon which was source tracked to the strawberries that were harvested from fields contaminated with deer feces [4]. Few studies have reported the prevalence of food borne pathogens such as *E. coli* O157 and *Salmonella* on fresh strawberries [5,6]. This shows that there is a need to analyze the microbial quality of fresh strawberries sold in markets. The microflora of hygiene indicators in food is strongly associated with the presence of food borne pathogens. One of the main groups of hygiene indicators is coliforms which includes lactose fermenting food borne pathogens [7]. Various methods are available to enumerate the coliform count but their efficiency vary with different category of samples. Hence, the two popular methods for coliform detection were analyzed with respect to fresh strawberries. Based on this background literatures, this study was designed to answer the question whether the strawberries sold in supermarkets are microbiologically safe to consume or not.

Materials and methods

Sample collection

The strawberries packed in punnet boxes were purchased from supermarkets at different locations in the west zone of Coimbatore, Tamil Nadu. Two punnet boxes were collected from each supermarket. Each punnet box weighed around 100g to 250g with nearly 7 to 13 strawberries based on the size of the berries. The punnet boxes were transported to the lab in ice box within 1 hr of collection and stored at 4°C until the further analysis which was commenced in an hour.

Sample preparation

The punnet boxes were opened in laminar air flow chamber and 25g of strawberries were weighed and transferred to stomacher bag (Baglight®, Interscience, France) in which 225ml of sterile peptone water (HiMedia, India) was added and blended in stomacher (BagMixer®, Interscience, France) for 2 minutes at the speed of 1 stroke per second. Then the mixture was serial diluted with sterile peptone water and used for microbiological analysis.

Microbiological analysis

The Total Aerobic Mesophilic microbial (TAM) count, fungal count, Enterobacteriaceae count were determined by serial dilution and plating technique in their respective medium such as Total Plate Count agar, Dichloran Chloramphenicol Rose Bengal agar and Violet Red Bile Glucose agar respectively based on International Organization for Standardization (ISO) [8-10]. The plates were incubated at 37˚C in incubator with the incubation period of 24 hours for total aerobic mesophilic and Enterobacteriaceae colony count and 3 to 4 days for fungal count.

Coliforms count by MPN and petrifilm

For MPN method, 1 ml from three consecutive dilutions were inoculated in three sets of tubes containing Lauryl Tryptose (LST) broth with inverted Durham's tube and incubated at 37˚C in incubator for 24-48 hours. The coliforms ferment the lactose in LST broth and result in gas production. The confirmation test for coliforms was performed by inoculating from the positive tubes of LST to the Brilliant Green Lactose Bile (BGLB) broth and the inoculated tubes were incubated at 37˚C in incubator for 48 hours [11]. For the enumeration of fecal coliforms, the LST positive tubes were inoculated in *Escherichia coli* (EC) broth tubes containing inverted Durham's tubes and incubated at 44˚C in incubator for 48 hours. The MPN index was calculated based on the positive tubes in each dilution from the MPN table and expressed as MPN/g of strawberries. For the petrifilm method, 1 ml of the appropriate dilution was inoculated in 3M petrifilm EC (3M™ Petrifilm™ EC, St. Paul, MN, USA) and incubated at 37˚C for 24 hours. The pink colonies with gas bubbles were counted as coliforms.

***E. coli* enumeration and *Salmonella* detection**

The presumptive *E. coli* was estimated by inoculating from the EC positive tubes to tryptone water and incubated at 44 ±1˚C for 48 hours. After the incubation period, the Kovac's reagent was added, shaken and allowed to stand for 5 minutes. The formation of red lake on top indicated the indole formation which shows the presence of presumptive *E. coli* [12]. The confirmation was done by streaking from the indole positive tubes on Eosine Methylene Blue (EMB) agar and observing for the colonies with green metallic sheen after 24 hours of incubation at 37˚C. In the petrifilm method, the blue colonies with gas bubbles were counted as *E. coli*.

Enrichment based method [13] was followed for detection of *Salmonella* in which the non-selective pre-enrichment was done in peptone water for 24 hours followed by selective enrichment in Rappaport Vassiliadis medium with soya (RVS) broth at 41.5˚C for 24 hours. After selective enrichment, the same was streaked on selective media namely Xylose Lysine Deoxycholate (XLD) agar and *Salmonella Shigella* (SS) agar and observed for black centered red colonies and black centered colorless colonies respectively after the incubation period of 24 hours at 37˚C. The positive colonies were pure cultured on nutrient agar and confirmed by the biochemical tests such as urease test and Triple Sugar Iron (TSI) test. The urea agar slants were prepared by adding filter sterilized 40% urea solution to the autoclaved urea agar. The isolates were streaked on urea slants and TSI slants followed by incubation at 37˚C for 24-48 hours. The typical *Salmonella* isolate is urease negative and shows red slant with acid butt in TSI slant accompanied with or without gas production and hydrogen sulphide production.

Statistical analysis

All the experiments were performed with six technical replicates. The cfu counts were converted to log10 cfu values and the statistical analysis was performed in SPSS Version 16.0. One-way ANOVA was performed followed by Duncan Multiple Range Test (DMRT) at significance level of $p \leq 0.05$. The MPN index was calculated based on number of positive tubes in each dilution according to ISO 7218 at 95% confidence interval. The index was taken along with its upper and lower limits and converted into log10 MPN. The value 0 was converted to 1 so that log10 value arrived at zero.

Results and Discussion

Total Aerobic Mesophilic microbial count (TAM), Fungal count and Enterobacteriaceae colony count

The Total Aerobic Mesophilic microbial count (TAM) was found to be significantly higher in samples collected from supermarkets 3 and 5 [Fig-1]. It was higher than the previously reported study [14] but the estimated fungal count range was slightly lower than the reported range of 2.10 – 5.86 log10 cfu/g by the same. The sample from supermarket 7 showed the highest fungal count [Fig-2]. The Enterobacteriaceae colony count was highest in supermarket 4 sample of nearly 4 log10 cfu/g [Fig-3] which was less when compared to the result reported earlier for the fresh strawberries procured from farms with and without organic amendments [15].

Coliforms

Since the MPN result is estimated count with its own upper and lower limits, it is difficult to compare quantitatively with other methods that enumerate the actual cfu count in the sample. So based on a study, the petrifilm count falling in the limit of corresponding MPN was considered to be equivalent [16]. In this study, all the log10 cfu counts of petrifilm method was found to be within the corresponding log10 MPN range [Fig-4] given by ISO [17]. The coliforms count was found to be in accordance with the previous study [14,18] and the highest count was recorded in market 1 sample in both MPN and petrifilm methods. Since the coliforms have diverse niche from feces and soil to the plant surfaces, the indigenous coliform population in plant surfaces may contribute for high level of coliform count apart from cross contamination. But in case of supermarket 1, the highest coliform count was associated with the fecal coliform count of 1 log10 MPN. Samples from supermarkets 3 and 5 had less than 1 log10 MPN of fecal coliforms. This shows the fecal contamination in strawberries which may be due to direct contamination of produce with feces or indirectly via contaminated soil, irrigation water or poor handling practices [19].

Table-1 *Locations where samples (strawberry punnet boxes) were collected*

Sampling locations in west zone of Coimbatore district	Supermarkets	Total number of punnet boxes collected
P.N. pudur	Supermarket 1	2
R.S puram	Supermarket 2 and 3	4
Sai Baba colony	Supermarket 4	2
Sukrawar pettai	Supermarket 5	2
Thadagam	Supermarket 6	2
Vadavalli	Supermarket 7 and 8	4

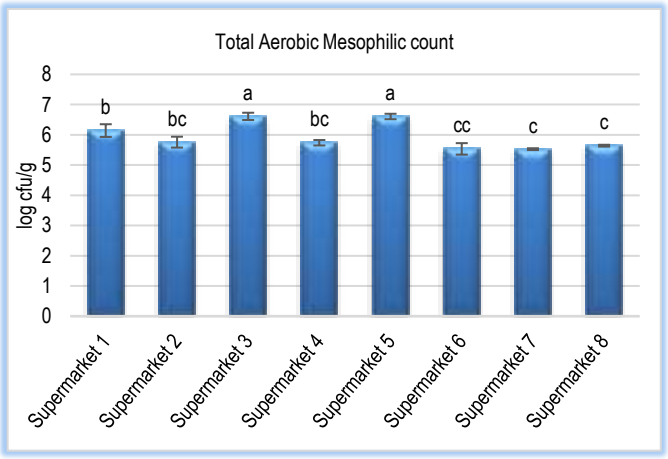

Fig-1 The mean TAM count of four replicates for each sample with standard error bar. The bars followed by same letter are not significantly different from each other at p≤0.05 determined by DMRT

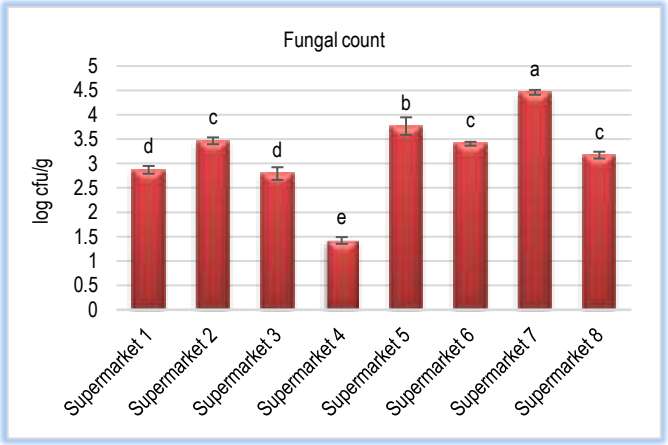

Fig-2 The mean fungal count of four replicates for each sample with standard error bar. The bars followed by same letter are not significantly different from each other at p≤0.05 determined by DMRT

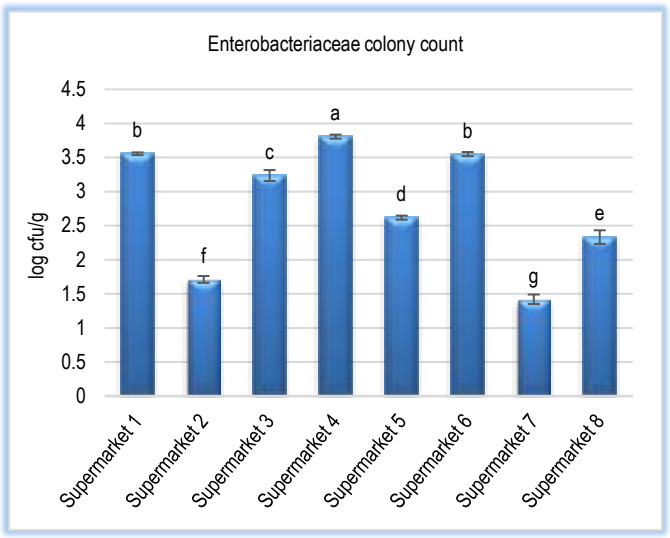

Fig-3 The mean Enterobacteriaceae colony count of four replicates for each sample with standard error bar. The bars followed by same letter are not significantly different from each other at p≤0.05 determined by DMRT

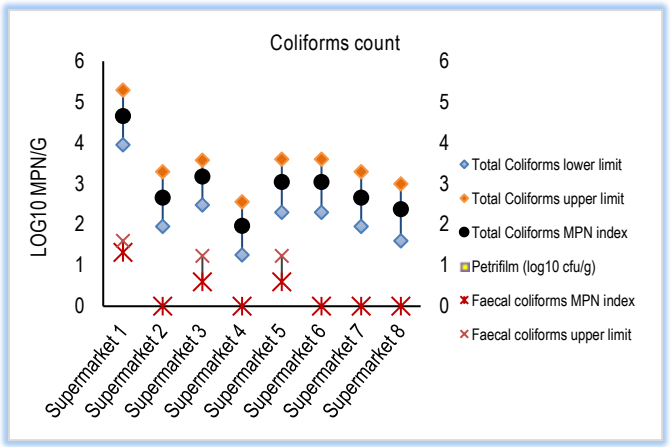

Fig-4 The total coliforms count was estimated by petrifilm and MPN methods. The fecal coliforms count was estimated by MPN method. The MPN index and limits are based on ISO 7218 at 95% confidence interval [17S]

E. coli and Salmonella

Though the strawberry samples have high coliform counts, they do not have *E. coli* and *Salmonella*. The absence of these important food borne pathogens in fresh strawberries were reported in some previous studies [14,18,20] but the potential risks associated with the high pathogen contamination in the components of primary production chain of strawberries have high probability to be transmitted to the produce which should not be left unmonitored [2,20]. In contrary, some of the studies have reported the presence of *E. coli* in high numbers in fresh strawberries [21]. The survival of *E. coli* O157 both on the surface and inside the fresh strawberries at varied storage temperatures had shown that their contamination in the farm to fork pathway of fresh strawberries can pose high risk to the human health [22].

The supermarket fresh strawberries were procured from different sources and the farming practices adopted by the farmers also influence the microbiological quality of fresh strawberries which is the consequence of devoid of washing step. Even if they are safe and not exposed to hazard till the point of harvest, there is a possibility of cross contamination in the marketing chain which may occur while packaging, transporting or handling in markets based on the hygienic practices followed in these stages [21,22]. Hence the detection of any hazard in the fresh produce is difficult to be traced back to the sources as the point of contact of produce and hazard vary both spatially and temporally.

Conclusion

The fresh strawberries in supermarkets does not have food borne bacterial pathogens but the high load of coliforms and fecal coliforms in some samples show that the samples were exposed to fecal contamination which may be the result of poor hygiene while handling or poor agricultural practices in primary production of strawberries. Hence, this work shows that food production and supply chain of strawberries should have stringent food protection measures to prevent exposure to potential microbial hazards.

Application of research: This work creates awareness about the microbial food safety of fresh fruits

Research Category: Food microbiology

Abbreviations: ANOVA: Analysis of Variance BGLB: Brilliant Green Lactose Bile, CFU: Colony Forming Units, EC: *Escherichia coli*
GAP: Good Agricultural Practices, GHP: Good Hygienic Practices
ISO: International Organization for Standardization
LST: Lauryl Sulfate broth, MPN: Most Probable Number
TAM: Total Aerobic Mesophilic microbial count, TSI: Triple Sugar Iron

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University: Tamil Nadu Agricultural University, Coimbatore, 641003, India
Research project name or number: MSc Thesis-Development of strategies for enhancing the quality and safety of strawberries from greenhouse to the market

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: west zone of Coimbatore, Tamil Nadu

Cultivar / Variety / Breed name: Strawberries (*Fragaria* X *Ananassa*)

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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