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Effects of Dipping Chicken Breast Meat Inoculated with *Campylobacter jejuni* in Turmeric Powder added Water

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A B S T R A C T

A study was conducted to assess the effect of immersing chicken breast meat inoculated with *Campylobacter jejuni* in turmeric powder added water. For this experiment, the chicken breast meat was portioned into pieces and sterilized by autoclaving. The sterilized chicken meat pieces were independently inoculated with standard *Campylobacter jejuni* culture at a concentration of 10^8 cells per gram of chicken meat and kept undisturbed for 30min at room temperature under aseptic condition to allow the bacteria to attach. The inoculated chicken pieces were randomly divided into six groups, each containing three pieces. Breast meat pieces from four groups were separately immersed in a glass beaker containing 20ml of 10%, 16.67%, 23.33% and 30% of turmeric powder added water for 15min, 15min, 5min and 5min, respectively. The fifth and sixth groups were dipped in 20ml of sterile distilled and tap water for 15min, respectively and maintained as controls. The breast meat pieces belonging to control and different treatment groups were individually drained, packed in sterile polyethylene bags, labelled and stored under refrigerated condition (4±1°C) for five days. The control and treated breast meat pieces were analyzed 45min after the treatments as well as on 3rd and 5th day of refrigerated storage for their effect on inoculated *Campylobacter jejuni*. The mean *Campylobacter jejuni* count of 16.67%, 23.33% and 30% turmeric powder added water treated chicken samples were significantly lower ($p<0.05$) from that of tap water and distilled water dipped control samples. Further, among turmeric water treated samples, 30% turmeric treatment resulted in significantly lower ($p<0.01$) *Campylobacter jejuni* count compared to all the other treatments studied.

Introduction

Raw chicken have been a significant sources of the bacterial pathogens like *Salmonella* and *Campylobacter* spp. (Deming *et al.,* 1987; Madden *et al.,* 2011; Drinceanu *et al.,* 2013). *Campylobacter* infections have been linked to poultry in many outbreaks (Tauxe *et al.,* 1988; Edwards *et al.,* 2014; Hope *et al.,* 2014; Lahti *et al.,* 2016), primarily due to the consumption of raw or undercooked chicken. Thermophillic *Campylobacter* spp. particularly *Campylobacter jejuni* and *Campylobacter coli* have emerged as major cause of diarrhoeal illness globally and are also responsible for the mortality and morbidity among children. In developed countries, *Campylobacter* spp. has been found to be second leading cause of diarrhea/gastrointestinal illness after *Salmonella* spp. Among human beings, *Campylobacters* have also been reported to cause severe conditions like Guillian Barre Syndrome, Chinese paralytic syndrome, hepatitis, meningitis and reactive arthritis (Allos, 1997; Korman *et al.,* 1997; Tenkate and Stafford, 2001). In India, several studies have revealed moderate to high prevalence of *Campylobacter* spp. in humans and livestock including poultry and their products (Raja, 2012; Rajendran *et al.,* 2012; Geetha, 2013; Mukherjee *et al.,* 2013; Dhanalakshmi *et al.,* 2014; Rajagunalan *et al.,* 2014; Begum *et al.,* 2015; Vaishnavi *et al.,* 2015).

Vast majority of the intervention technologies that include trimming (Prasai *et al.,* 1995a,b), water washing (Hardin *et al.,* 1995; Reagan *et al.,* 1996), hot water spraying (Smith and Graham, 1978), steam pasteurizing (Phebus *et al.,* 1997), as well as sanitizing by chemicals such as organic acids (Hardin *et al.,* 1995; Dorsa *et al.,* 1997), chlorine compounds (Kotula *et al.,* 1974) and polyphosphates (Dickson *et al.,* 1994) etc., for decontamination of red meat and poultry carcasses have been developed and some were patented by the research institutions in developed countries. However, these technologies could be integrated as a unit operation in 'on the rail dressing' performed by large and medium scale modern abattoir to reduce the likelihood of pathogenic microorganisms being present on the carcasses and meat. In developing countries like India, scattered meat production system and lack of modern slaughter facilities to cater the domestic market restrict the application of such intervention technologies at the meat processing environment. Hence, in addition to the implementation of hygienic principles of meat processing, developing and adopting a decontamination technology that could be applied by the consumer at household level would definitely validate the safe consumption of meat by the consumers to a greater extent.

Turmeric powder is being used as an ingredient in almost all the Indian meat preparations. Several researchers have documented the antimicrobial and antioxidant properties of turmeric (Goel, 2007; Lourenco *et al.,* 2013; Deshmukh, 2014; Pandey *et al.,* 2014; Rachana and Venugopalan, 2014; Gupta *et al.,* 2015; Mohammed and Habil, 2015). It is noteworthy to mention that immersing/washing meat in/with turmeric powder added water prior to cooking is a household practice in India. Keeping above facts in consideration, this study is proposed to assess the efficacy of dipping chicken breast meat inoculated with *Campylobacter jejuni* in turmeric added water.

Materials and Methods

Collection of chicken meat samples

The chicken breast meat samples were collected within 3 to 4h of slaughter, packed individually in clean, sterile polyethylene

bags and transported to the Food and Industrial Microbiology Laboratory at College of Food and Dairy Technology, Koduvalli, Chennai, in an insulated container maintained at 4±1°C under hygienic condition and utilized for the study.

Chemicals, Media, Buffers and Reagents

All the chemicals used in the study were of analytical grade purchased from reputed national and international firms. Media, supplements and laboratory aids used in the study were procured from HiMedia and Oxoid.

Standard culture (*Campylobacter jejuni*)

Standard culture of *Campylobacter jejuni* for inoculation study was procured from Division of Veterinary Public Health, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India. The culture was tested for its purity, morphological and biochemical characteristics and then, maintained by sub culturing in Brain Heart Infusion broth.

Turmeric Powder

The dried turmeric rhizomes purchased from the market were adjusted to the moisture content of less than 12% using solar drier and ground into fine powder in a spice pulverizer. The turmeric powder obtained was employed for preparation of different concentrations of turmeric powder added water and the later were used in this study as decontaminant.

Selection of contact times for different concentrations of turmeric powder added water

In this trial, the contact times for chicken breast meat samples in four different concentrations of turmeric powder added water were separately standardized by sensory evaluation. For this purpose, turmeric powder were individually dissolved at a rate of 15g, 25g, 35g and 45g for every 150 ml of sterile distilled water in order to prepare four different concentrations (10%, 16.67%, 23.33% and 30%) of turmeric powder added water.

To standardize the contact time for each concentration, about 150g of chicken breast meat samples were individually immersed in 600ml of each concentration of turmeric powder added water, at 1:4 (W/V) ratio, for three different contact times *viz.*, 5, 10 and 15 min. After specified period of immersion, the chicken breast meat samples were removed, drained and kept in clean plates. 45min after the treatment, the samples were cooked to the internal temperature of 72°C and the intensity of effect of immersion in different concentration of turmeric powder added water on colour, odour, taste and overall acceptability of the chicken breast meat samples were subjectively evaluated against a nine point hedonic scale (Meilgaard *et al.*, 2006) by a sensory evaluation panel comprising students and staff of College of Food and Dairy Technology, Koduvalli, Chennai. The maximum contact time at which each concentration of turmeric powder added water had minimal or no effect on sensory attributes was chosen. Accordingly, one contact time was selected for each concentration of turmeric powder. By the end of this preliminary trial, four different concentration-contact time combinations were obtained and utilized in further study.

Effect of turmeric powder added water on *Campylobacter jejuni* inoculated over chicken breast meat samples

The inoculation study was carried out to compare the decontaminating efficacy of different concentration-contact time combinations of turmeric powder added water

over *Campylobacter jejuni* inoculated on chicken breast meat pieces. The effect of treatments was assessed 45min after the treatment as well as on day three and five of refrigerated storage (4±1°C).

Preparation of Standard *Campylobacter jejuni* culture for inoculation studies

The standard *Campylobacter jejuni* culture was freshly inoculated in BHI broth and grown overnight at 37°C. The cells were made into pellet by centrifugation at 8000rpm for 10min and washed twice with sterile phosphate buffered saline (PBS). The final cell pellet was suspended in PBS and the concentration of cells was adjusted to 10^8 cells/ml using McFarland's nephelometer tubes.

Inoculation in chicken breast meat

Chicken breast meat obtained from retail market was hygienically portioned into pieces of five grams. Then, eighteen such pieces were individually wrapped in aluminum foil and sterilized by autoclaving at 121°C (15lbs pressure) for 15min. The sterilized chicken meat pieces were independently inoculated with *Campylobacter jejuni* culture at a concentration of 10^8 cells per gram of chicken breast meat and kept undisturbed for 30min at room temperature to allow the bacteria to attach. All the procedures were carried out aseptically to avoid any contamination.

Immersion in turmeric powder added water

The inoculated chicken breast meat pieces were randomly divided into six groups, each containing three pieces. Breast meat pieces from four groups were separately immersed in a glass beaker containing 20ml of 10%, 16.67%, 23.33% and 30% turmeric powder added water for their respective contact times selected through preliminary trial. The fifth and sixth groups were dipped in 20ml of sterile distilled and tap water for 15min, respectively and maintained as controls. The breast meat pieces belonging to control and different treatment groups were individually drained, packed in sterile polyethylene bags, labelled and stored under refrigerated condition (4±1°C) for five days. The control and treated breast meat pieces were analyzed 45min after the treatments as well as on 3rd and 5th day of refrigerated storage for their effect on inoculated *Campylobacter jejuni*. On each such occasion of analysis, one chicken breast meat sample was drawn from each of the control and treatment groups.

Enumeration of *Campylobacter jejuni*

28.35g of Park and Sander's broth base and 2% of agar was suspended in 940ml of sterile distilled water, boiled to dissolve completely and sterilized by autoclaving at 121°C (15lbs pressure) for 15min. Final pH was adjusted to 7.0±0.2. Prior to pouring into the petridishes 5% of sterile defibrinated lysed sheep blood, 10ml of reconstituted contents of Park and Sander's selective supplement I and II were aseptically added. The contents were mixed well and maintained at 44-46°C. Approximately, 10-15ml of sterilized media containing supplements was poured into the sterile petridishes under aseptic conditions for preparation of plates in order to inoculate the aliquots. Spread plate method was followed for inoculation of 0.1ml aliquots of suitable sample dilutions. Inoculated plates were incubated for 48h at 42-43°C under micro-aerophilic conditions. The typical colonies of *Campylobacter jejuni* were counted and expressed as $\log_{10}$cfu/g of sample.

Statistical analysis

Data collected were analyzed using standard statistical procedures (Snedecor and Cochran,

1994). Analysis of Variance (ANOVA) procedure was used to determine the significant difference ($p \leq 0.05$) among the means obtained for different treatments. The results of storage studies were analyzed by two-way analysis to know the effect of treatments and storage period.

Results and Discussion

Standardization of contact times for different concentration of turmeric powder added water based on sensory attributes

As discussed in materials and methods section 2.5, series of preliminary trials were conducted to identify optimum contact time for each of the four different concentrations of turmeric powder added water used in this study. The contact times so chosen are summarized in the Table 1.

With respect to chicken breast samples treated with 10% as well as 16.67% turmeric powder added water, mean sensory scores for colour, odour, taste and overall acceptability did not significantly differ ($p>0.05$) between the contact times studied. The mean colour and odour scores of 23.33% turmeric powder added water treated chicken breast samples did not significantly differ ($p>0.05$) between the contact times whereas mean scores for taste and overall acceptability were significantly higher ($p<0.05$) for 5 min than 15min contact times. Similarly, mean colour and odour scores of chicken breast samples treated with 30% turmeric powder added water did not significantly differ ($p>0.05$) between contact times but the mean scores for taste and also overall acceptability were significantly higher ($p<0.05$) for 5min than 15min contact times.

Based on the statistical inferences, the contact time for dipping chicken breast samples in 10%, 16.67%, 23.33% and 30% turmeric powder added water were determined as 15min, 15min, 5min and 5min, respectively.

Effect on inoculated *Campylobacter jejuni*

Effect of dipping inoculated chicken breast meat pieces in selected concentration and contact time combinations of turmeric powder added water on *Campylobacter jejuni* count, as affected by refrigerated storage (4±1°C), are presented in Table 2.

The overall treatment mean values for *Campylobacter jejuni* count (Fig. 1) of 16.67%, 23.33% and 30% of turmeric powder added water treated chicken breast meat samples significantly differed ($p<0.05$) from tap water and distilled water dipped control samples whereas 10% turmeric treated samples were significantly lower from that of tap water dipped control ($p<0.05$**)** but not with distilled water dipped control samples ($p>0.05$). Among turmeric water treated samples, the mean *Campylobacter jejuni* count did not significantly differ ($p>0.05$) between 16.67% and 23.33% turmeric treatments as well as between 10% and 16.67% turmeric treatments. The mean level of reductions in *C.jejuni* count (from initial inoculation level of 10^8 cells per gram of meat) by different concentration-contact time combinations of turmeric powder added water treatments varied between 2.35 and 2.53 $\log_{10}$cfu/gram of sample. However, control samples treated with sterile distilled water and tap water also showed approximately 2.25 $\log_{10}$cfu/gram of sample.

Finally, the results of the present study showed that 30% turmeric powder added water treatment resulted in significantly higher reduction in *Campylobacter jejuni* count compared to other treatments used as well as controls.

Table.1 Standardization of contact times for different concentrations of turmeric powder added water based on sensory attributes (Mean±SE)[#]

Standardization of contact time for 10% turmeric powder added water			
Sensory attributes	**Contact times**		
	5 min	10 min	**15 min**
Colour	6.77±0.20	6.91±0.21	6.91±0.19
Odour	6.83±0.24	7.00±0.20	6.93±0.18
Taste	7.06±0.18	7.22±0.17	6.83±0.18
Overall acceptability	7.04±0.18	7.10±0.18	6.83±0.16
Standardization of contact time for 16.67% turmeric powder added water			
Sensory attributes	**Contact times**		
	5 min	10 min	**15 min**
Colour	7.25±0.18	7.41±0.14	7.06±0.18
Odour	7.14±0.17	7.41±0.15	7.18±0.15
Taste	7.20±0.13	7.29±0.16	6.81±0.19
Overall acceptability	7.29±0.12	7.41±0.13	7.06±0.15
Standardization of contact time for 23.33% turmeric powder added water			
Sensory attributes	**Contact times**		
	5 min	10 min	15 min
Colour	6.91±0.14	6.93±0.15	6.81±0.20
Odour	7.18±0.16	7.12±0.16	6.85±0.17
Taste	6.77±0.16 [a]	6.22±0.21[ab]	5.77±0.23[b]
Overall acceptability	7.00±0.17 [a]	6.77±0.20[ab]	6.35±0.21[b]
Standardization of contact time for 30% turmeric powder added water			
Sensory attributes	**Contact times**		
	5 min	10 min	15 min
Colour	6.37±0.22	6.18±0.24	6.12±0.25
Odour	6.72±0.16	6.58±0.21	6.37±0.19
Taste	5.75±0.26[a]	5.06±0.28[ab]	4.87±0.31[b]
Overall acceptability	6.20±0.27[a]	5.77±0.28[ab]	5.33±0.29[b]

- Average of six trials.

The mean scores in a row with similar superscripts or no superscript represents that there is no significant difference.

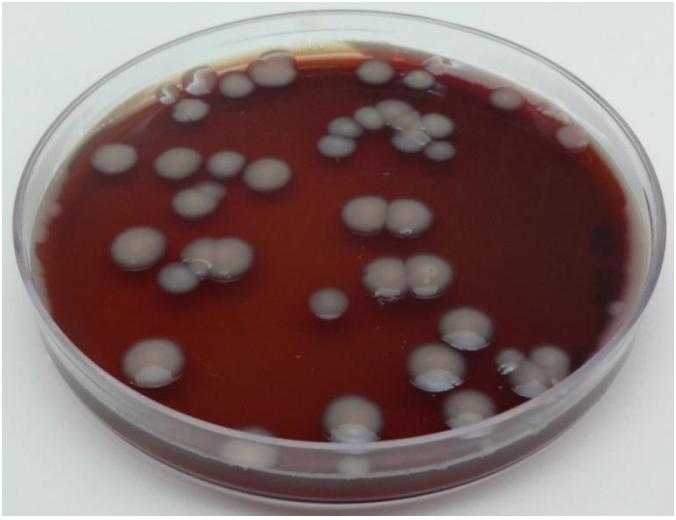
The mean scores in a row with different superscripts represents that there is significant difference.

Table.2 Effect of turmeric powder added water on *Campylobacter jejuni* inoculated chicken breast meat samples at different storage period (Mean±SE)[#]

Effect on *Campylobacter jejuni* count							
Storage period	**Control [log(cfu/g)]**		**Treatment [log(cfu/g)]**				**Overall Mean**
	DW	**TW**	**T1**	**T2**	**T3**	**T4**	
Day 0	6.79±0.07	6.83±0.06	6.72±0.05	6.65±0.05	6.60±0.05	6.44±0.05	**6.67±0.03 A**
Day 3	5.83±0.05	5.84±0.04	5.71±0.04	5.68±0.04	5.64±0.04	5.58±0.04	**5.71±0.02 B**
Day 5	4.55±0.04	4.59±0.05	4.51±0.04	4.48±0.04	4.44±0.04	4.40±0.04	**4.40±0.02 C**
Overall Mean	**5.72±0.22[a] b**	**5.75±0.22 a**	**5.65±0.22 bc**	**5.60±0.22[c] d**	**5.56±0.22 d**	**5.47±0.20[e]**	**5.63±0.09**

- Average of six trials
DW – Distilled water treated chicken breast meat pieces (control 1)
TW – Tap water treated chicken breast meat pieces (control 2)
T1 – 10% Turmeric Powder added water treated chicken breast meat pieces
T2 – 16.67% Turmeric Powder added water treated chicken breast meat pieces
T3 – 23.33% Turmeric Powder added water treated chicken breast meat pieces
T4 – 30% Turmeric Powder added water treated chicken breast meat pieces
The mean scores in a row with similar superscripts or no superscript represents that there is no significant difference.
The mean scoresin a row with different superscripts represents that there is significant difference.

Fig.1 Petri dishes showing *Campylobacter jejuni* colonies recovered from turmeric water dipped inoculated chicken breast meat samples.



However, the difference in level of reduction observed between 30% turmeric powder added water treatment and control(s) was approximately 0.3 log_{10}cfu/g of sample. Similar results was observed by Goel *et al.*, (2007)who found that *Campylobacter jejuni*

was resistant to hexane extracts of turmeric while studying bio-protective properties of turmeric.

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