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Effectiveness of Microwave Radiation in the Inactivation of Coliforms Present in Jarjeer (*Eruca sativa*)

Dr. Arturo L. Tapas Jr.
College of Engineering, AMA International University, Bahrain

Abstract: The number of total coliforms is widely used as indicator of the presence of pathogens. They coexist in the human large intestine and even after disposal to the environment. One useful application is in the determination of the presence of coliforms in food, specially those to be consumed by the public without further heat treatment or cooking. Such is the case with ready to eat (RTE) salads. In this study, the vegetable jarjeer (*Eruca sativa*), rocket in English, was the vegetable tested. Jarjeer is usually a component of green salads and there were reports that these vegetables were harbouring coliforms which could not be removed by washing. It was therefore appropriate to test some household common disinfection method such as microwaving if it would be effective in inactivating these coliforms present in jarjeer. Both the time of inactivation and the thickness of the jarjeer placed in the plate were varied in the experiment. At a thickness of 0.1 cm and inactivation time of 15 seconds a 58% reduction in coliform was obtained and at 30 seconds inactivation time 81 % reduction was obtained. At a thickness of 1 cm, no reduction of coliforms was obtained at 15 and 30 seconds inactivation time. Note that the thickness of 0.1 cm was equivalent to the single leaf of vegetable placed on the plate so that it is already the minimum thickness possible for the study. A 58 or 81 % inactivation of coliforms is not acceptable as far as health safety is concerned since there are still plenty of coliforms left in the leaves. Ideally there should be few or no coliforms present (99.9 % inactivation). We therefore conclude that microwaving is not an effective method to inactivate the coliforms in jarjeer.

Keywords: coliforms, vegetable salads, pathogen, microwave, inactivation.

1. INTRODUCTION

Nowadays, people are very much health conscious and this is the reason that there is now a large demand for ready to eat (RTE) salads. These are being sought by people due to their nutrients as well as low-calorie content.

But the same people who are health conscious may not be knowledgeable about the possibility of bacterial contamination of these raw vegetable salads. These raw salads do not undergo the cooking process thus any bacterial contamination will tend to stay in the salad or worse, grow in the salad at an exponential rate of growth if the environmental conditions are favorable.

These vegetables undergo a lot of processes and handling before finally reaching the end consumers so that it is very likely that bacterial contamination will take place. Well of course there is always the possibility that some of these bacteria are actually pathogenic, since fecal contamination from the soil as well as from the food handlers may always happen.

It is therefore advisable that these bacteria present in the salads be inactivated to prevent the occurrence of diseases related to the fecal contamination of salads. Here, microwaving is tested as to its effectiveness in the disinfection of salads. There is a possibility of the salads getting cooked during microwaving, this problem can be minimized by reducing the exposure time to microwaves.

The presence of coliforms in any material is always taken to be indicative of fecal contamination and because of the ease in the determination of coliforms, this has been used as indicators of pathogens in the food since pathogens are largely coming from the feces of people. The inactivation of coliforms in food will also indicate that any pathogens present will be consequently inactivated.

The most probable method (MPN) method of coliform was used in assessing the level of coliforms in the water. It does not need complicated equipment and the laboratory procedure is very simple. It offers a convenient procedure for the monitoring of coliform levels and thus the possibility of a real risk to pathogen contamination can be evaluated and prevented.

1.1 Statement of the Problem

Ready to Eat (RTE) salads are now very popular among health-conscious people and there is a need to protect these people from the possibility of being infected by fecal microorganisms that are may be pathogenic. When food is

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contaminated by human feces, it will always be assumed that it is not safe for human consumption because of the inherent nature of feces to harbor pathogens.

1.2 Objectives:

(1) To determine the effectiveness of using microwave in the inactivation of coliforms present in RTEs.
(2) To determine the effect of inactivation time and salad thickness in the effectiveness of microwaving
(3) To use MPN method in the enumeration of total coliforms.

1.3 Scope and Limitation

In this study, the MPN method was used in the detection and enumeration of coliforms. Microbial counts were enumerated by MPN method because it could be performed in the laboratory with common glasswares like test tubes, autoclave and incubator. The study gave a range of detection based on MPN three dilutions and triplicates for each dilution. Jarjeer, a vegetable used in salads which were not cooked, was the focus of this study.

2. LITERATURE REVIEW

2.1 Ready to Eat Salads (RTEs) and Food Borne Diseases

RTEs are becoming more popular nowadays because people are becoming more health conscious and more educated when it comes to nutrition. These RTEs are usually eaten raw and consequently outbreaks of diseases traceable to presence of coliforms are now prevalent.

The contamination of food by coliforms indicates a very dangerous situation since they are usually existing with other pathogenic entero bacteria. There are many diseases that can be attributed to this entero bacteria which are the same diseases that are found in the water which are referred to as water borne diseases. A comprehensive compilation of these food-borne diseases and the organisms causing can be found in the USA Food and Drug Administration [4].
In Pachuca, Mexico, samples of mung bean sprouts were found to contain fecal coliforms, Salmonella and pathogenic variety of *E. coli*. Most RTE salads were found to be unsafe due the presence of coliforms that can cause illness [5].
In the work of Pereira et al.[6], it was found that the working conditions affected the microbiological quality of RTEs, and recommendations were given involving the washing of the salads with chlorine tablets. In a study conductedf in Turkey, a similar situation was recorded. E. coli and other pathogenic microorganisms were detected in RTEs. These microorganisms even exhibited resistance to antibiotics [7].

In a study conducted by O'Connor et al [8], fecal coliforms and other pathogens were detected in fresh mangoes and puree cheeks. They recommended that pasteurization be applied to make it safe for human consumption.
The contamination of food by coliform indicates a very dangerous situation since they are usually existing with other pathogenic entero bacteria. There are many diseases that can be attributed to these entero bacteria which are the same diseases that could originate from contaminated water (water borne diseases). A comprehensive compilation of these food-borne diseases and the organisms causing them can be found in the USA Food and Drug Administration [4].

2.2 Source of Contamination in the Vegetable Production Chain

2.2.1 Transmission of Human Pathogens in Manure, Water and Soil

This is the transfer of pathogens through the fertilizers used, irrigation water which may be contaminated by human wastes and the soil itself which may have the natural micro flora of coliforms [13]. In poor countries of Asia, agricultural lands are still irrigated by untreated sewage water [14]. There is therefore a very high risk of pathogen contamination from vegetables grown in these areas.
There is study made suggesting that a minimum of 90 days should be allowed to reduce the coliform content in the soil-sludge mixture by 90%. It is suggested that vegetables should be planted only 90 days after the application of sludge to the soil [15].

2.2.2 Handling of the Food Before and After Harvesting

This is contamination of pathogens from the way vegetables are handled. For example, equipment used in packaging may be contaminated and the people handling the vegetables may also transfer pathogens into the vegetables [16]. A good example of this is the uprooting and bundling of the jarjeer greens in Sharjah which is being done by hand [17]

2.2.3 Processing of the Vegetables

Equipment used to further process the vegetables into products for distribution may be contaminated.

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2.2.4 Growth and Death of Pathogens Within the Vegetables

Some pathogens may grow and accumulate in the vegetable parts, others may die.

2.3 Indicator Organisms

The coliform organisms are used as the indicator organisms because of the following characteristics: (1) they are not themselves pathogenic so that they are not dangerous to handle during bacteriological analyses, (2) they die more slowly than pathogenic organisms so that absence of coliforms may mean absence of pathogens and (3) it is easy and not expensive to analyze for coliforms [13].

Coliforms may come from humans and animals. Fecal coliforms die very fast outside the intestine so that the presence of fecal coliforms indicates a recent contamination. Total coliforms and fecal coliforms are related to each other by the following [18].

(1) Total coliforms has many subgroups.

(2) Fecal coliforms fall under total coliforms and they may also come from other sources like animals not only people.

(3) *E. coil* falls under fecal coliforms and they come from mammals. *E. coli* is very good indicator of contamination from people but they may also come from other mammals.

2.4 Nature of Microwaves

Microwaves are one class of electromagnetic radiation with 0.001 to 1.000 m in wavelength and 300 to 300,000 MHz in frequency [1,2]. Micro in microwaves does not mean that the wavelengths are in the micrometer order of magnitude but it means that the wavelengths are shorter than the radiowaves.

Microwaves' operates in a frequency that enable it to pass through materials while converting its electromagnetic energy into the deeper part of the materials. In that case not only the surface of the material will be heated. The frequency of commercially available microwave ovens for kitchen use is 2.45 GHz, while the frequency for maximum absorbance by water is approximately 10 GHz [2]. We see that this is not optimize for water to make deeper food penetration. Otherwise, only the surface will absorb the radiation and become very hot and the deeper part still cold.

2.5 Inactivation of Microorganisms Using Microwaves

The inactivation of Microorganisms by microwaves is due to dielectric heating. In the study by conducted by Dong [12], it was observed that microwave radiation was effective in the inactivation of microorganisms present in kitchen sponges, syringes and scrubbing pads. The presence of ions in the water can increase the effectiveness of heating and subsequent disinfection effect of microwaves [3].

Microwaves have been used to inactivate microbes in sludge with the final application of H_2O_2. The purpose of H_2O_2 was to prevent regrowth of microbes in the sludge. After the process the sludge was observed to be free of microorganisms [9]. Even though it was sludge that had been disinfected in this study it does suggests that micowaves are effective in the inactivation of microbes and pathogens and might be used in the disinfection of food.

In the study by Mun [10], it was found that microwaves were more efficient than ultraviolet and ozone treatment in the inactivation of Ascaris eggs.

Jalapeno peppers subjected to microwaves at a maximum temperature of 63 ºC was found to have a 4-5 log reduction removal in Salmonella [11]. Immediately the peppers were cooled to 4 ºC to minimize the effect on the freshness. Discolorization was observed but the hardness of the peppers remained the same.

3. RESEARCH METHODOLOGY

3.1 General Approach to the Conduct of the Study

The objective of this research is to determine the reduction of coliforms in jarjeer after being subjected to microwaving, the dependent variable was the effectiveness of microwaving in the reduction of coliforms in the vegetable salads. The independent variables were be time of microwaving and thickness of the vegetable salads exposed to micowaves. In order not to appreciably affect the crispiness of the vegetables after microwaving, only short period of contact times were used, 15 and 30 seconds.

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3.2 Sampling Preparations of Samples and Analysis

Samples were taken from restaurants selling vegetable salads including jarjeer. The numbers of coliforms were enumerated before and after microwaving.

In the microbial analyses of samples, it was necessary that interferences in the analyses were eliminated. These interferences were mainly from contamination coming from the use of equipment and glasswares. Contamination from the air could also contribute to the errors in the analysis.

To prevent contamination, sterilization of glasswares was done by using autoclave. Sterilization was confirmed by utilizing a negative control. The negative control was composed of the solution media except the sample. This would

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determine if sterilization of the media and water used in the study was complete before being used. All equipment used and all the plastic bags used in sampling were sterilized prior to being used. It was also insured that the laboratory was cleaned by disinfectants everyday and that air circulation was minimized inside the laboratory.
The samples were analyzed for coliforms without washing since these were ready to eat salads. The number of coliforms per gram of the sample was computed before and after microwaving.

3.3 Microbial Methods

The most probable number of coliforms (MPN) was determined using a presumptive test in accordance with the standard methods.
The salad vegetables to be analyzed was first homogenized using an electric blender. A sample from the salad was mixed with 200 mL sterilized water. Then 1 mL of this preparation was diluted by adding 9 mL of the prepared medium Lauryl Tryptose Broth. This was the first dilution tube and labeled as dilution 0.1. Dilutions 0.01 and 0.001 were subsequently prepared from this using sterilized water. It was assumed that in the preparation of the dilutions, there was perfect mixing and the coliforms were then randomly distributed. A 24-hour incubation period followed. The number of positive samples was counted by noting the development of turbidity and production of bubbles in the durham tubes. Tapping of the tubes would released bubbles and would make them more visible. The number of positive tubes in each dilution was noted and the MPN determined from the table. Calculation of the result was expressed in MPN per gram of vegetable sample.

4. RESULTS AND DISCUSSIONS

4.1 MPN Coliform Counts of the Vegetable Before Microwaving

The concentration of jarjeer mixed in the water was set as 10 grams per 200 mL of water. Positive tubes combination 3-3-2 was obtained. The number of coliforms was computed to be 22,000 CFU/g. Table 4.1 gives the details of the result.

TABLE 4.1 10 GRAMS OF JARJEER IN 200 mL WATER, THICKNESS OF SAMPLE, BEFORE MICROWAVING

Positive tubes		
0.10 dilution	**0.01 dilution**	**0.001 dilution**
3	3	2
MPN = 1100 CFU/ mL or 22,000 CFU/g		

4.2 MPN Coliform Counts of the Vegetable After Microwaving

The concentration of jarjeer mixed in the water was set as 10 grams per 200 mL of water. Potive tubes combination of 3-3-1 was obtained . The number of coliforms was computed to be 9200 CFU/g. Table 4.1 gives the details of the result.

TABLE 4.2 10 GRAMS OF JARJEER IN 200 mL WATER, THICKNESS OF SAMPLE 0.1 cm AFTER 15 MINUTES MICROWAVING

Positive tubes		
0.10 dilution	**0.01 dilution**	**0.001 dilution**
3	3	2
MPN > 1100 CFU/ mL or 92,000 CFU/g 58 % inactivation		

4.3 MPN Coliform Counts of the Vegetable After Microwaving

The concentration of jarjeer mixed in the water was set as 10 grams per 200 mL of water. Positive tubes combination of 3-3-1 was obtained. The number of coliforms was computed to be 9200 CFU/g. Table 4.1 gives the details of the

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TABLE 4.3 10 GRAMS OF JARJEER IN 200 mL WATER, THICKNESS OF SAMPLE 0.1 cm AFTER 30 MINUTES MICROWAVING

Positive tubes		
0.10 dilution	**0.01 dilution**	**0.001 dilution**
3	3	1
MPN > 210 CFU/ mL or 4,200 CFU/g 81 % inactivation		

The above tables are typical and repeatable. It is enough to show that even in the 81 % inactivation of coliforms there will still be plenty of coliforms left in jarjeer to be considered safe for human consumption. Inactivation should be 99.99% which means very few coliforms are left in the vegetable.

5. CONCLUSIONS AND RECOMMENDATIONS

It is readily apparent that microwaving of the vegetable is not enough to reduce to safe levels the number of coliforms of jarjeer. To insure safety in the consumption of food, it is necessary to reduce the number of coliforms in the vegetable by 99.9% equivalent to a few remaining bacteria in the vegetable. This would eliminate the risk of pathogen infection and diseases. From the data gathered in the study, it can be concluded that microwaving is not a practical and effective way of eliminating the pathogens in the vegetable.

It does not mean however that the presence of coliforms in vegetable or salad being consumed by the people indicates 100% contamination by pathogens since there are other sources of coliforms. The leaves of the vegetable are good hiding places for microorganisms but they are not necessarily pathogenic.

But the presence of substantial number of colifroms clearly indicates that microwaving is not effective in the elimination of coliforms in the raw salad vegetables, and therefore it does not reduce the risk of pathogen infection if there is a pathogen contamination in the vegetable.

It is recommended that to prevent the occurrence food-borne diseases from ready to eat salads, these vegetables should be grown in a supervised and controlled garden so that there is no possibility of pathogen contamination.

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