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Comparison of Escherichia coli Identification in Chicken Meat by Impedance and Conventional Culture

Z. Zadbakhshi Seighalani[1], A. Sharifan[2], M. Hashemi Ravan[3]*

[1,2] Department of Food Science and Technology, Science and Research Branch Islamic Azad University, Tehran, Iran.

[3] Department of Food Science and Technology, Varamin – Pishva Branch, Islamic Azad University, Varamin, Iran

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ABSTRACT: Due to the resistance of Escherichia coli to environmental conditions and its high density in food, which indicates the unfavorable health status of food preparation and distribution centers, the count of Escherichia coli in poultry meat and compliance with the existing standards, including routine work it is a laboratory. On the other hand, the speed of achieving results as soon as possible is one of the special points to be considered in order to ensure the quality of the product. Therefore, based on this issue, the use of impedance technique was considered as a new method in assessing the presence of Escherichia coli in chicken meat, which allows less time to achieve results faster. In this study, four samples of raw chicken, processed chicken, grilled heated chicken and oven-heated chicken with two reference and impedance methods were used to separate Escherichia coli. The impedance technique was performed based on recording the results every ten minutes from measuring the electrical resistance changes of the liquid culture medium (M-Value) used in this method. Then, using SPSS software, the compatibility of the two methods was compared. By comparing these two methods with each other, it was shown that there was no significant difference between the results of these two methods in investigating the presence of Escherichia coli in these samples. According to the results obtained in terms of the importance of obtaining results as soon as possible in food quality control tests, the use of new techniques such as impedance method in the food industry can be used as an alternative to conventional methods.

Keywords: *Chicken meat, Escherichia coli, Food quality, Impedance, M value*

INTRODUCTION

Chicken meat is of special importance in nutrition due to the higher percentage of protein than other meats, low drop after slaughter, high digestibility, high growth rate of chicken and its low price compared to other meats, and on the other hand Protein production by raising chickens is simpler, easier and more feasible than other livestock. For these reasons, chicken meat has been widely used as a food source of protein in human nutrition around the world. After slaughtering

(*) Corresponding Author - e-mail: azadeh.zadbakhsh@gmail.com

poultry, a series of chemical, physical and microbial changes begin in the carcass and meat, which result in a significant reduction in the quality characteristics of the product. Product quality assessment methods are mainly designed based on the progress of these changes, including changes in microbial density and growth of various microorganisms [1]. In the last two decades, the use of electrical resistance or impedance method to identify important microbes in food and also the general identification of microbes in various foods has become widespread. In this method, rapid detection of bacteria is possible by displaying metabolic activity by altering the electrical resistance in the culture medium [2]. The presence of Escherichia coli is one of the most important factors in evaluating the quality of various foods. Determining the presence of Escherichia coli in poultry meat and processed products such as sausages and conforming the results with the values of existing standards, including common laboratory techniques in all factories as well as food hygiene monitoring departments. On the other hand, the speed of achieving test results as soon as possible and in the shortest possible time is one of the special points considered by factories as well as food hygiene monitoring departments in order to ensure the quality of manufactured products. Therefore, in the present study, the use of impedance technique to evaluate the presence of Escherichia coli in raw, processed and heated chicken (oven and grilled) and the consistency of the results with the results of the reference method of pour plate was considered.

In general, the impedance method is based on placing special impedance vials containing the test fluid inside the greenhouse under the desired thermal conditions, which over time due to the metabolism of microorganisms, nutrients in the culture medium that has a higher molecular weight and ionic load. Are less, broken down into metabolites with lower molecular weight and higher ionic load. Therefore, the ability to move and conduct more electricity between the electrodes in the special vial of the impedance method shows that it indicates a decrease in impedance or apparent resistance in the system. These changes are measured and recorded by special vial electrodes of the impedance method. Microorganisms (in the case of using specific culture media) are identified based on the duration and the amount of changes in impedance and consequent electrical conductivity of the set. Also, according to the type of microorganisms, counting or calculating their number is possible with the help of device software, which is especially used in the recent case of general microbial counting in food (ISIRI No. 7727, 2004) [3-5].

MATERIALS AND METHODS

In the present study, the presence of Escherichia coli in samples of raw chicken, processed chicken, grilled chicken and oven-heated chicken was investigated. Tests for Escherichia coli were performed on 10 samples of each treatment in three replications, a total of 120 samples.

First, raw materials were purchased from the market and divided into four categories. Part of it was sent directly to the laboratory as a sample of raw chicken meat. The other part was processed with different spices and formed a sample of processed chicken. Some of the samples were grilled and the other part was heated in the oven. Finally, the samples were tested by two standard methods using impedance to examine the presence or absence of Escherichia coli in them.

Conventional culture method

First, 1 g of the sample is added to 10 ml of Ringer's solution to obtain the initial suspension. 10 ml of the initial suspension will be added to 10 ml of the tube containing the double concentration of lauryl sulfate. Inoculated tubes containing double lauryl sulfate will be placed at 37 °C for 24 hours. If no gas or turbidity is observed at this stage, incubation will continue for up to 48 hours. If any gas or turbidity is observed in the tubes containing double lauryl sulfate after incubation, it will be removed by the culture ring and inoculated into the EC broth culture medium and incubated in a hot water bath or 44 °C incubator for 24 hours. If no gas is observed in the pipes at this stage, the heating time will last up to 48 hours. After this period, if gas is observed in the tubes, these tubes will be inoculated into the peptone water environment by the culture ring and incubated at a temperature of 44 degrees Celsius for 48 hours. After 48 hours, 0.5 ml of coax reagent is

added to the tubes containing inoculated peptone water and mixed well and checked after one minute. The formation of a red ring in the alcohol phase proves the presence of an indole ring. The presence of indole ring also indicates the presence of Escherichia coli in the volume or weight of the test (ISIRI No. 2946 - Second revision, 2005) [6].

Cultivation by impedance culture method directly

First, the double lauryl sulfate culture medium is made and sterilized according to the instructions on the culture medium, and then it is divided into 9 ml volumes in special sterile impedance vials. They can also be sterilized after dividing the culture medium into special impedance vials. Then 1 ml of diluted food sample is poured into the vial. The vials are placed in the mentioned cavities inside the greenhouse of Back Truck 4300 microbial analyzer (manufactured by Sy-lab Company) in such a way that they are properly placed in the cavities and ensure that the electrodes of the vials are connected to the electrodes at the end of the cavities. Tube specifications including type and sample number are entered in the device software and the protocol related to the evaluation of Escherichia coli at 37 degrees Celsius using changes in impedance or electrical resistance in culture medium (M-value) with an initial heating time of 1 hour and a limit 5% threshold and impedance measurement intervals equal to every 10 minutes are set for a maximum duration of 24 hours of device operation and the results of measuring the amount of impedance changes and consequently electrical conductivity are recorded in the software of Buck Truck 4300 microbial analyzer. The results of detecting and counting Escherichia coli by reference method and also the time obtained to evaluate the microbial load in terms of hours by the impedance device are recorded in the special software system of Buck Truck 4300 microbial analyzer designed based on Excel and The correlation curve of the two methods with the highest coefficient of determination (R2) is obtained and based on this, the formula or equation of the relevant regression curve, which is for predicting and mathematically calculating microbial density based on the impedance time parameter, is obtained [7].

For statistical analysis of this study, using SPSS software and Kruskal-Wallis test, an in-group comparison between the presented results was performed. Then the frequency and frequency percentage in each group were determined by conventional method and impedance method. Finally, the results obtained were compared through the Whitney-Mann U test to determine the significant difference between the results of the conventional method and the impedance method.

RESULTS AND DISCISSION

Similar research has been done by researchers to compare the impedance method with the reference method in search of microbes in different foods, including the study of Fazl ara et al. In 2012 that mathematical modeling of microbial density based on the impedance method in Poultry fillets were evaluated. They evaluated 80 samples of poultry meat fillets using the pour plate reference method and the impedance method. The compliance rate of these two methods was reported to be 97.4% and it was stated that the use of new techniques such as the impedance method in the food industry as an alternative to the old conventional methods, can be done [7].

Fallah et al. conducted a comparative study on impedance and reference methods in identifying enterococci contamination in food. In fact, the purpose of this study was to use the reference method and evaluate its compliance with the impedance method in order to achieve a more accurate and faster method that can evaluate the tested samples in a shorter time. In this study, the reference method was performed ISIRI No. 2198 entitled "Search, identification and counting of intestinal enterococci in food." The impedance technique was performed based on recording the results every ten minutes once by measuring the electrical resistance changes of the liquid culture medium (M-value) used in this method. Then, using SPSS software, the degree of compliance of the two methods was compared. Finally, it was stated that the impedance method can be used as an alternative method in terms of high compliance of 83.3% with the reference method in detecting enterococcal infection [8,9].

Bancalari et al. worked on the use of impedance to accelerate monitoring of exopolysaccharides from

lactic acid bacteria. Bacterial exopolysaccharides have been considered due to their different properties in manufacturing, biotechnology and nutrition industries. The direct monitoring method of exo-polysaccharides is enriched based on the study of filamentous tissues of colonies developed on solid culture media. To overcome the weaknesses of this direct monitoring method, in this study, impedance was used to evaluate the exo-polysaccharide produced by lactic acid bacteria in milk. The results obtained in this study showed that the impedance method overcomes the limitations of the direct method and can be introduced as a suitable substitute for it [10,11].

Jasem et al. used an impedance biosensor to simultaneously detect low concentrations of Salmonella in poultry and fresh foods such as lettuce. The goal was to detect Salmonella rapidly and simultaneously in these products. This biosensor had a limit of 7 CFU/mL for detection. The biosensor also had the ability to selectively detect Salmonella in the presence of other pathogens, as well as to distinguish between living and dead cells [12].

During the study conducted in this study, the presence of Escherichia coli in samples of raw chicken, processed chicken, grilled chicken and oven-heated chicken was investigated. Tests for Escherichia coli were performed on 10 samples of each treatment in three replications, a total of 120 samples.

First, raw materials were purchased from the market and divided into four categories. Part of it was sent directly to the laboratory as a sample of raw chicken meat. The other part was processed with different spices and formed a sample of processed chicken. Some of the samples were grilled and the other part was heated in the oven. Finally, the samples were tested by two standard methods using impedance to examine the presence or absence of Escherichia coli in them. To compare raw chicken in both methods, three replications within each group were first compared to determine whether there was a difference in the three repetitions between the groups. The following are the results of the Kruskal-Wallis test.

As can be seen in Table 1, the intragroup comparison shows that there is no significant difference between the three repetition times in the conventional method and in the impedance method ($p < 0.05$). In other words, the results were similar in three repetitions.

As can be seen in Table 2, about 30% of the raw chicken meat-conventional method was negative and about 70% was positive.

As can be seen in Table 3, about 40% of the raw chicken meat-impedance method was negative and about 60% was positive. Raw chicken meat was repeated three times by conventional method and impedance and compared in general to determine wheth-

Table 1. Results of Kruskal-Wallis test to compare three repetitions within a group

Raw chicken meat - impedance method	Raw chicken meat - the usual method	
0.806	0.00	Chi-square
2	2	Degrees of freedom
0.668	1.00	Significance level

Table 2. Frequency and frequency percent of raw chicken meat in the usual method

Cumulative frequency	Frequency percent	Frequency		
30.0	30.0	9	Negative	Chicken
100.0	70.0	21	Positive	Raw- the
	100.0	30	Sum	usual Method

Table 3. Frequency and frequency percent of raw chicken meat in the Impedance method

Cumulative frequency	Frequency percent	Frequency		
40.0	40.0	12	Negative	Chicken
100.0	60.0	18	Positive	Raw
	100.0	30	Sum	Impedance Method

Table 4. Results of the Whitney-Mann U test to compare three repetitions within a group

Sum	Repetition 3	Repetition 2	Repetition 1	
405.00	45.00	40.00	50.00	Whitney-Mann U
870.00	100.00	95.00	105.00	Wilcoxon
0.805	0.457	0.890	0.00	Z
0.421	0.648	0.374	1.00	Significant Level

Table 5. Frequency and percentage of raw chicken meat - the usual method is repeated three times

Sum	Repetition					1
			3			2
9	3	3	3	Frequency	Negative	Raw chicken meat - the usual method
100.0%	33.3%	33.3%	33.3%	percent		
21	7	7	7	Frequency	Positive	
100.0%	33.3%	33.3%	33.3%	percent		
30	10	10	10	Frequency		Sum
	100.0%	33/3%	33.3%	33.3%		percent

er there is a significant difference between the two groups or not.

As the results of the Whitney-Mann U test show, there is no significant difference between the three repetitions and the total score of the two groups of raw chicken meat by conventional method and impedance. In other words, there is no significant difference between the two groups.

The results remained unchanged in three replications. The frequency and percentage of raw chicken meat-impedance method were examined in three repetitions.

To compare processed chicken meat in both methods, three replications within each group were first compared to determine whether there was a difference in the three repetitions between the groups.

As can be seen in Table 7, the in-group comparison shows that there is no significant difference between the three repetition times in the conventional method and in the impedance method ($p < 0.05$). In other words, the results were similar in three repetitions.

As can be seen in Table 8, about 20% of processed

Table 6. Frequency and percentage of raw chicken meat-impedance method in three repetitions

Sum	Repetition					1
			3			2
12	4	5	3	Frequency	Negative	Raw chicken meat - Impedance method
% 100.0	33.3%	41.7%	% 25.0	percent		
18	6	5	7	Frequency	Positive	
100.0%	33.3%	27.8%	% 38.9	percent		
30	10	10	10	Frequency		Sum
	100.0%	33.3%	33.3%	33.3%		percent

Table 7. Results of Kruskal-Wallis test to compare three repetitions within a group

Raw chicken meat - impedance method	Raw chicken meat - the usual method	
1.160	0.00	Chi-square
2	2	Degrees of freedom
0.560	1.00	Significance level

Table 8. Frequency and percentage of frequency of chicken processed in the usual way

Cumulative frequency	Frequency percent	Frequency		
20/0	20/0	6	Negative	Chicken
100/0	80/0	24	Positive	Raw - the
	100/0	30	Sum	Usual Method

Table 9. Frequency and percentage of frequency of chicken processed by impedance method

Cumulative frequency	Frequency percent	Frequency		
33/3	33/3	10	Negative	Chicken
100/0	66/7	20	Positive	Raw - Impedance
	100/0	30	Sum	Method

Table 10. Results of the Whitney-Mann U test to compare three repetitions within a group

Sum	Repetition 3	Repetition 2	Repetition 1	
390.00	40.00	40.00	50.00	Whitney-Mann U
855.00	95.00	95.00	105.00	Wilcoxon
1.158	0.951	0.951	0.00	Z
0.247	0.342	0.342	1.00	Significant Level

Table 11. Frequency and percentage of frequency of processed chicken meat - the usual method is repeated three times

Sum	Repetition				1	
			3		2	
6	2	2	2	Frequency	Negative	Processed chicken meat - the usual method
100.0%	33.3%	33.3%	33.3%	percent		
24	8	8	8	Frequency	Positive	
100.0%	33.3%	33.3%	33.3%	percent		
30	10	10	10	Frequency	Sum	
	100.0٪	33.3٪	33.3٪	33.3٪	percent	

Table 12. Frequency and percentage of frequency of processed chicken meat – Impedance method is repeated three times

Sum	Repetition				1	
			3		2	
10	4	4	2	Frequency	Negative	Processed chicken meat - Impedance method
100.0%	40%	40%	20%	percent		
24	6	6	8	Frequency	Positive	
100.0%	30%	30%	40%	percent		
30	10	10	10	Frequency	Sum	
	100.0%	33.3%	33.3%	33.3%	percent	

chicken - the conventional method - was negative and about 80% was positive.

As can be seen in Table 9, about 33% of processed chicken meat - the impedance method - was negative and about 67% was positive. Conventionally processed chicken meat and impedance were repeated three times and compared in general to determine whether there is a significant difference between the

two groups.

As the results of the Whitney-Mann U test show, there is no significant difference between the three repetitions and the total score of the two groups of chicken processed by conventional method and impedance. In other words, there is no significant difference between the two groups. Then the frequency and frequency of processed chicken meat-conventional method was examined in three repetitions. The results remained unchanged in three repetitions.

To compare grilled chicken meat in both methods, three repetitions within each group are first compared to determine if there is a difference in the three repetitions between the groups.

As can be seen in Table 13, the in-group comparison shows that there is no significant difference between the three repetition times in the conventional method and in the impedance method ($p < 0.05$). In other words, the results were similar in three repetitions.

As can be seen in the table above, 100% of grilled chicken is a typical negative method.

As can be seen in Table 15, 100% of grilled chicken is impedance-negative. Then, the grilled chicken meat was repeated three times in the usual way and impedance and compared in general to determine whether there is a significant difference between the two groups or not.

As the results of the Human Whitney test show, there

Table 13. Results of Kruskal-Wallis test to compare three repetitions within a group

Grilled chicken meat - impedance method	Grilled chicken meat - the usual method	
0.00	0.00	Chi-square
2	2	Degrees of freedom
1.00	1.00	Significance level

Table 14. Frequency and percentage of frequency of chicken processed by the usual method

Cumulative frequency	Frequency percent	Frequency		
100	100	30	Negative	grilled chicken meat - the usual method

Table 15. Frequency and percentage of frequency of chicken processed by impedance method

Cumulative frequency	Frequency percent	Frequency		
100	100	30	Negative	grilled chicken meat – Impedance method

Table 16. Results of the Whitney-Mann U test to compare three repetitions within a group

Sum	Repetition 3	Repetition 2	Repetition 1	
450.00	50.00	50.00	50.00	Whitney-Mann U
915.00	105.00	105.00	105.00	Wilcoxon
0.00	0.00	0.00	0.00	Z
0.0001	1.000a	1.000a	1.000a	Significant Level

Table 17. Frequency and percentage of frequency of grilled chicken meat by the usual method

Sum	Repetition				1	
			3		2	
100	10	10	10	Frequency	Negative	grilled chicken meat - the usual method
100.0%	33.3%	33.3%	33.3%	percent		
30	10	10	10	Frequency	Sum	
	100.0%	33.3%	33.3%	33.3%	percent	

Table 18. Frequency and percentage of frequency of grilled chicken meat by Impedance method

Sum	Repetition					
			3			1
						2
100	10	10	10	Frequency	Negative	grilled chicken meat - the usual method
100.0٪	33.3٪	33.3٪	33.3٪	percent		
30	10	10	10	Frequency		Sum
	100.0٪	33.3٪	33.3٪	33.3٪		percent

Table 19. Results of Kruskal-Wallis test to compare three repetitions within a group

Heated chicken with oven – the impedance method	Heated chicken with oven - the usual method	
0.000	0.000	Chi-square
2	2	Degrees of freedom
1.000	1.000	Significance level

Table 20. Frequency and percentage of frequency of heated chicken with oven by the usual method

Cumulative frequency	Frequency percent	Frequency		
100	100	30	Negative	heated chicken with oven - the usual method

Table 21. Frequency and percentage of frequency of heated chicken with oven by Impedance method

Cumulative frequency	Frequency percent	Frequency		
100	100	30	Negative	heated chicken with oven - Impedance method

is no significant difference between the three repetitions and the total score of the two groups of grilled chicken meat by conventional method and impedance. In other words, there is no significant difference between the two groups.

The frequency and percentage of grilled chicken meat - impedance method is repeated three times.

To compare heated chicken with an oven in both methods, three repetitions within each group were first compared to determine whether there was a difference in the three repetitions between the groups. The following are the results of the Kruskal-Wallis test.

As can be seen in Table 19, the intragroup comparison shows that there is no significant difference be-

Table 22. Results of the Whitney-Mann U test to compare three repetitions within a group

Sum	Repetition 3	Repetition 2	Repetition 1	
450.00	50.00	50.00	50.00	Whitney-Mann U
915.00	105.00	105.00	105.00	Wilcoxon
0.00	0.00	0.00	0.00	Z
0.0001	1.000a	1.000a	1.000a	Significant Level

Table 23. Frequency and percentage of frequency of grilled chicken meat by the usual method

Sum	Repetition					
			3			**1**
						2
100	10	10	10	Frequency	Negative	heated chicken meat with oven - the usual method
100.0٪	33.3٪	33.3٪	33.3٪	percent		
30	10	10	10	Frequency		Sum
	100.0٪	33.3٪	33.3٪	33.3٪		percent

Table 24. Frequency and percentage of frequency of grilled chicken meat by the usual method

Sum	Repetition			1		
			3	2		
100	10	10	10	Frequency	Negative	heated chicken meat with oven - Impedance method
100.0%	33.3%	33.3%	33.3%	percent		
30	10	10	10	Frequency	Sum	
	100.0%	33.3%	33.3%	33.3%	percent	

tween the three repetition times in the conventional method and in the impedance method ($p < 0.05$). In other words, the results were similar in three repetitions.

As can be seen in Table 20, 100% of the chicken heated by the conventional oven is negative.

As can be seen in Table 21, 100% of the chicken meat heated with the oven-impedance method is negative. Then, the chicken meat heated with the oven in the usual way and impedance was repeated three times and compared in general to determine whether there is a significant difference between the two groups or not.

As the results of the Human-Whitney test show, there is no significant difference between the three repetitions and the total score of the two groups of heated chicken meat with the oven by conventional method and impedance. In other words, there is no significant difference between the two groups.

CONCLUSIONS

In the present study, the identification of Escherichia coli in raw chicken, processed chicken, grilled heated chicken and oven-heated chicken by conventional and impedance methods was investigated. In raw chicken meat, about 70% of the samples were positive in the conventional method and 60% of the samples were positive in the Escherichia coli impedance method. By comparing these two methods with each other, it was shown that there was no significant difference between the results of these two methods in the study of raw chicken meat. In the case of processed chicken, about 80% of the samples were positive in the conventional method and 67% of the samples were positive in the Escherichia coli impedance method. By comparing these two methods with each other, it was shown that there was no significant difference between the results of these two methods in the study of processed chicken meat. In the case of grilled chicken, 100% of the samples were negative in the conventional method and 100% of the samples in the Escherichia coli impedance method were negative. Comparing these two methods with each other, it was shown that there was no significant difference between the results of these two methods in the study of grilled chicken. In the case of oven-heated chicken, 100% of the samples were negative in the conventional method and 100% of the samples in the Escherichia coli impedance method were negative. Comparing these two methods with each other, it was shown that there was no significant difference between the results of these two methods in the study of heated chicken meat with an oven. In the end, it can be concluded that the impedance method is a suitable and efficient method to replace the identification of Escherichia coli bacteria in the usual way.

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AUTHOR (S) BIOSKETCHES

Z. Zadbakhshi Seighalani, Department of Food Science and Technology, Science and Research Branch Islamic Azad University, Tehran, Iran, *Email: azadeh.zadbakhsh@gmail.com*

A. Sharifan, Department of Food Science and Technology, Science and Research Branch Islamic Azad University, Tehran, Iran

M. Hashemi Ravan, Associate Professor, Department of Food Science and Technology, Varamin – Pishva Branch, Islamic Azad University, Varamin, Iran