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Comparison of impedance splitting method to pour plating method for the estimation of bacterial count in mayonnaise

[1*]Faraji, S., [2]Fazlara, A., [3]Hashemi Ravan, M., [1]Faraji, N. and [1]Taheri, Sh.

[1]*Department of Food Science, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran*
[2]*Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran*
[3]*Department of Food Science , Pishva Branch , Islamic azad University,Varamin,Iran*

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Abstract

The objective of the present study was to employ the technique of Impedance Splitting Method, a technique of high accuracy and sensitivity, in the microbial count and characterization in the shortest possible time. A total bacterial count on samples (n = 100), collected during the winter and summer season, were evaluated by the standard and the Impedance splitting methods (ISM). Based on the obtained coefficient of determination results in the resulting mathematical equations, the correlation coefficient between the two methods in relation to the total bacterial count in mayonnaise, in the summer and winter seasons, as well as the cumulative results have been 93.2, 88.4 and 91%, respectively. The time required to obtain these result were 19.66 hours for the summer and the cumulative samples and 5.12 hours for the winter samples. Comparison to the conventional method of pour plating, time is shortened from 1-3 days to only a few hours.

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Introduction

Impedance Splitting Method (ISM) is a relatively rapid technique employed for the quick detection and characterization of microorganisms based on their rate of metabolic activity leading to a change in electrical resistance. In addition, Impedance is the basis for the invention of such analyzing instruments as Bactrometer, Maltus and Bac Tract (Firstenberg-Eden, 1983).In the process of microbial metabolism, the nutrient substrates containing macromolecules like proteins, peptides and carbohydrate are used up, converted into smaller micro-molecules, and electrically charged by products. These byproducts can change the electric conductivity of liquid nutrients and thereby reduce the substrates impedance. This reduction is measurable with two electrodes (Silley and Forsythe, 1996).

Impedance measurement has been used for quality control in food industries and especially in identification, counting of indicator microorganisms and of estimation of antimicrobial activity (Fontana *et al.*, 2002; Gerolimatou *et al.*, 2004; Batrinou *et al.*, 2005). In the past two decades, the use of Impedance for the detection and characterization of resistant microorganisms in various food products has been quite widespread. Therefore, the European Union’s office of Standards has developed the AFNOR, and ISO, for the use of Impedance over the more time consuming technique of pour plating. Numerous researches have been carried using the Impedance method in microbial quality control of food products.

Monitoring the total microbial loading of a wide range of food products has been evaluated and shown to be successful for frozen vegetables (Hardy *et al.*, 1977), grain products (Sorrells, 1981), UHT low acid foods (Coppola and Firstenberg-Eden, 1988), confectionery (Pugh *et al.*,1988), fish (Ogden,1986) and meat products (Firstenberg-Eden, 1983; Fletcher *et al.*, 1993; Russell *et al.*, 1994; Silley *et al.*, 1996). In the enumeration of total bacterial count, the microbial count of mesophiles in mayonnaise must not exceed what is mandated by the Institute of Standards and Industrial Research of Iran set guidelines, (ISIRI 2965, 2008). Furthermore, it is of utmost importance that the evaluation of high quality products can be carried out in the shortest amount of time. Therefore, as with the time consuming nature of pour plating technique, including the preparatory stages, the present study was carried out to compare the use of a newer technique, i.e., Impedance, with the existing standard model of pour plating. A predictive mathematical model designed for this comparison is presented. The aim of the present study was to investigate the reliability of the Impedance measurement for the estimation of microbial population on mayonnaise for the first of its kind carried out in the world.

Materials and Methods

Sample preparation

Totally 100 samples of mayonnaise were purchased during cold and warm seasons from

*Corresponding author.
Email: *Faraji_soh@yahoo.com*

market places in Ahvaz (a city in Khozestan province, Iran) and tested for total bacterial count (mesophile bacteria) by standard methods and impedance standard methods were based on references to standard institute and method. The investigation of Iran's recommendation and the impedance method was industrial measuring the variation of electrical resistance of broth media (M-value) done by electrodes (E-value) for determination of total bacterial. Experiments were performed in laboratory of Shahid Chamran University of Ahvaz (Ahvaz, Iran). The standard method was carried out by pour plate technique for total bacterial count according to the recommendations of standard methods. The impedance-splitting method was performed every 10 minutes measuring the variation of electrical resistance of Kanamycin Esculin Azide borth (M-value) which was used in this method and reversed to the electrical resistance, the electrical conductance curves were drawn by Bac Trac 4300 microbial analyzer instrument software.

Plate Count Method

Following the plate count agar sterilization, a mayonnaise (10 cc) – ringer (90 cc) mixture was prepared. 0.1-0.00001 mayonnaise dilutions were prepared and 1 cc of each dilution was transferred to their corresponding sterilized agar plates, followed by 10-15 ml addition of PCA, so that the bottom of the plates were covered. The agar was allowed to solidify at room temperature. After a couple of minutes, once again the plates were covered by a thin layer of PCA, 5 ml. Upon the solidification of the second layer of agar, the inverted plates were incubated at 37^{o}C for 72 hrs (ISIRI 2965, 2008). Subsequent to a 72 hrs incubation period, counting of the colonies with a colony counter began. The protocol set forth by the Institute of Standards and Industrial Research of Iran, (ISIRI 2325, 2002) was followed for the counting of the colonies. Data on the colony count is presented logarithmically, Log cfu.g^{-1}. The plate's colony counts, i.e. those with 30-300 colonies, were multiplied by the inverse dilution rate. The present study evaluated three replicates of each of the 5 dilutions (ISIRI 2325, 2002).

Impedance culture method

Similar procedures were followed using the SY-LAB microbial analyzer. The SY-LAB measurements are based on Impedance of and the electrical conductivity between the electrodes inside the 001A vials - impedance specific laboratory vials. First, 9 cc 001A growth medium vials were sterilizated for 15 minutes at 121^{o}C and 1.5 atm. Next, the growth medium was cooled down to 40-45^{o}C. Subsequently, under sterile conditions, 1 cc of the mayonnaise sample solutions were transferred to the vial. The vials were then placed in a 370C incubator for a period of 24 hrs. A total bacterial count using the impedance method was repeated three times on all test samples (ISIRI 7726 and 7727, 2008).

Statistical analysis

Data on the cumulative total bacterial count which were obtained through impedance and the reference methods, as well as the times obtained via Impedance method was recorded using the EXCEL software. The best correlation curve with the highest coefficient of determination (R^2) was obtained. Based on the data, the regression equation for the predictive mathematical calculation of the microbial load was designed. The mathematical calculations were based on the Impedance detection time (IDT). The obtained results, breaking down the summer and winter data, were analyzed using SPSS 16 software by the Independent T test, One Sample T test and the Mac Nemar analysis.

Results and Discussion

Total bacterial count: pour plating method

The present study examined the microbial load of the mesophile bacteria in mayonnaise samples taken during the summer and the winter season. Based on the total bacterial colony count results from the three replicates of each experimental run, the mean averages from the reference method were compared with those obtained from the impedance method. The highest contamination level in the summer and winter season were 1.23×10^8 cfu.ml^{-1} and 3.7 × 107 cfu.ml^{-1}, respectively. And, the lowest level of contamination in the summer season was reported as 3.3×10^2 and 8.3×10^3 in the winter season.

Total microbial count: impedance method

Subsequent to samples introduction to a calibrated impedance analyzer, time changes in impedance, IDT, were recorded, in less than 24 hrs. Calibration curves, correlation coefficients (r), and dispersion (SYX) parameters for calibration evaluation were determined. Summer samples' total microbial mean and standard error ($1.6 \times 10^7 \pm 5.21 \times 10^6$ cfu.ml^{-1}) as well as standard deviation (2.901×10^7) were obtained. Thus, the summer microbial load averages are more than the set standard limits for mayonnaise. There is a statistically significant difference between the summer microbial load and the standard limit 10^3 cfu.ml^{-1} ($p < 0.05$).Winter samples microbial

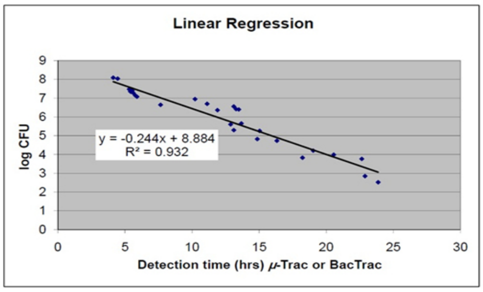

Figure 1. The IDT correlation test results with the summer mesophile bacterial count

Syx = 0.411
r = -0.9658

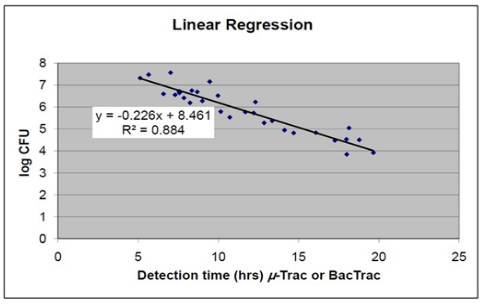

Figure 2. The IDT correlation test results with the winter mesophile bacterial count

Syx = 0.362
r = -0.9403

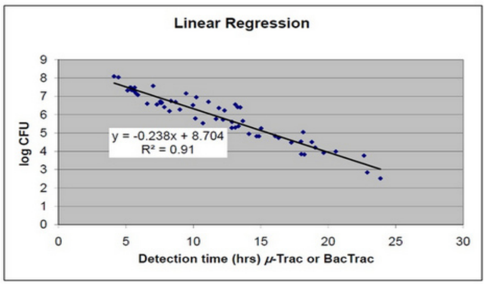

Figure 3. The IDT correlation test results with the cumulative mesophile bacterial count

Syx = 0.399
r = -0.9539

averages ($4.81\times10^6 \pm 1.601\times10^6$ cfu.ml^{-1}) and the corresponding standard deviation (8.9×10^6) were also obtained. The average microbial count of the winter samples were shown to be higher than the set standard limit and the statistical analysis of data exhibited a significant difference between these two values 10^3 cfu.ml^{-1} ($p < 0.05$).

The cumulative microbial averages ($1.04 \times 10^7 \pm 2.797 \times 10^6$ cfu.mil^{-1}) and the degree of standard deviation of samples (2.202×10^7) were determined. The cumulative microbial averages were shown to be over the standard limit set for mayonnaise sample and a statistically significant difference between the samples microbial count and that of the standard (10^3 cfu.ml^{-1}) was demonstrated ($p < 0.05$). The summer, winter and the cumulative Correlation curve of IDT and the reference method was obtained through the linear regression test. Thus, the bacterial regression equation (1) in summer season with the determined IDT by Bac Trac 4300 is as follows:

$$Y = -0.244X+8.884 \quad R^2 = 0.932 \quad \text{Equation 1}$$

The coefficient of determination is $R^2 = 0.932$, which shows the degree of correlation between the two methods. Thus, the impedance method can be employed for a quick calculation of (Y), microbial count results by placing the detection time values (X) in equation (1). The winter samples bacterial regression equation with determined IDT by Bac Trac 4300 is as follows:

$$Y = -0.226x+8.461 \quad R^2 = 0.884 \quad \text{Equation 2}$$

In the present study, a comparison of the total microbial count by impedance and reference method was carried out. The degree of correlation between the two methods was high for the winter sample, $R^2 = 0.884$. The cumulative (summer and winter) bacterial regression equation with determined IDT by Bac Trac 4300 is as follows:

$$Y = -0.238X+8.704 \quad R^2 = 0.91 \quad \text{Equation 3}$$

A coefficient determination of $R^2 = 0.91$, shows a 91% correlation between the two methods.

Impedance analyzer is a device of high value in the quality control of food products. It is, thereby, of great importance in the field of food science, for its quick, cost effective, and economic quantitative bacterial estimation and characterization, based on measuring the breakdown of nutrients by bacteria through changes in electrical impedance of the medium (Batrinou *et al.*, 2005) The pour-plating method used for the evaluation of 50 samples of mayonnaise, exhibited 31 (62%) contamination in the summer season samples, 29 of which (93.5%) exceeded the set maximum standard limit of contamination (10^3 cfu.ml^{-1}). The maximum and minimum amount of time taken to come up with these results, through impedance method, were 23.87 hrs and 4.12 hrs, respectively. These results indicate the advantage of the Impedance method over the standard Pour –Plating which takes 1-3 days.

The ideal correlation coefficient (r), and the dispersion value (SYX) are set between (-0.85 to -1.0) and 0.5, respectively. The present study's reported correlation coefficient (r = -0.9658) and the (SYX = 0.411) were shown to be less than the set values, therefore, the obtained curve is acceptable as the standard curve for the mayonnaise summer samples in the Khuzestan region of Iran. Thirty-one (62%) of mayonnaise samples (n = 50) taken at winter time exhibited microbial contamination, in 100% of which contamination (10^3 cfu.ml^{-1}) exceeded the set standard limit. Furthermore, the impedance was, once again, shown to be a faster technique over the standard pour plating, as the maximum and minimum amount of time required to obtain the results were shown to be 19.66 hrs and 5.12 hrs, respectively.

The correlation coefficient, (r = 0.9403), and the dispersion values, SYX = 0.362, were also less than the standard limit. Based on the obtained results, the resulting curve can be considered as the standard curve for mayonnaise in winter season in the Khuzestan region. Thus, the overall findings suggest that within limits of 2-8 logarithms, both contamination and degree of bacterial dispersion are greater in the summer season. We also obtained samples with the highest (1.23×10^8 cfu.ml^{-1}) and lowest (3.3×10^2 cfu.ml^{-1}) degree of contamination in this season. On the other hand, winter samples exhibited lower degree of contamination and dispersion, 3-7 logarithms, compared to the summer samples.

The observed seasonal difference in state of Khuzestan could be related to the difficulties in maintaining a cold environment during production, storage and distribution, so, the observed degree of contamination in this season (Log7). There is no doubt that microbial contamination would be less in the wintertime in all stages of production, storage and distribution (log 5). The wide distribution of microbial contamination data in the summer attributed to seasonal differences, and therefore, the impedance changes in this season corresponds to the obtained curve. The same results were reported by Firstenberg –Eden and Tricarico (1983) extended Impedance Splitting Method to the determination of mesophilic and psychotropic counts in raw milk.

The overall findings suggest that out of a total 100 mayonnaise samples, 62% have been contaminated; with 60 (96.8%) of these samples having a contamination level, (10^3 cfu.ml^{-1}) that exceeds the standard limit. The time required to obtain results through the impedance method also was faster than the standard pour plate method, i.e. 4.12 to 23.87 hrs vs. 1-3 days. The correlation between the two methods was equal to 91%. Finally, considering the dispersion value (SYX = 0.399) and the correlation coefficient (r = -0.9539) results the obtained data can be accepted as the standard values for mayonnaise produced in state of Khuzestan. Similar to our results obtained by (Fazlara *et al*., 2007) on pasteurized butters. They reported that, impedance-splitting method can be used as alternative techniques in microbial quality control of pasteurized butters in dairy industries.

Overall, the average microbial load was shown to be higher in the summer time. Statistical analysis of data also exhibited a significant difference between the curve equations and the mathematical models, ($P < 0.05$). Therefore, it is strongly recommended to differentiate between the use of these two models in winter and summer time. It is also recommended to use the specially obtained mathematical model for the separate calculation of mayonnaise samples microbial contamination in winter and summer seasons rather than the cumulative mathematical and the curve equation models. The results of the present study indicating the use of season specific mathematical model is confirmed by and are in line with the rationale behind the use of the Impedance technique. Similar results were obtained by other researchers (O'connor, 1979; Gnan and Luedecke, 1982; Nieuwenhof and Hoolwerf, 1987) established impedance-splitting method use for monitoring total bacterial counts in raw milk.

Researchers have previously carried out similar research involving a comparison of impedance method and that of standard pour plating for the estimation of bacterial contamination in foods. Lack (2006), carried out the comparative study of the two methods on ice cream samples (n = 50), with 80% correlation. The lower degree of observed correlation, much like the present study, can be attributed to variations in ice creams formulations. Batrinou *et al.* (2005) in yet another research involving the comparison of these techniques examined the mixed microbial population of bitter chocolate and presented a 89.89% correlation based on regression curve. The obtained correlation in Batrinou's study is quite similar to that of the present study, in terms of methodology, medium, and test conditions. In addition, dark chocolate samples had different consistencies like that of mayonnaise samples, which could have affected the results.

As in all the aforementioned studies, no statistically significant difference between the results of the two methods were observed. Furthermore, the minor variations in results can be attributed to such variables as the composition, type of bacteria, medium, microbial analysis, and laboratory personnel's errors and degree of accuracy. Similar to previous studies, the present study evaluated mayonnaise samples (n = 100) using the two methods of Impedance and pour plating. 60 percent of these samples showed contamination above the set standard limit. No statistically significant difference between the two methods was revealed using the Mac Nemar test, ($p > 0.05$). Thus, in light of these findings, the impedance method is presented as a better alternative to the standard method of pour plating in that it offers an easy, rapid quantitative bacterial estimation and detection time, as well as, greater quality control in the production of mayonnaise. This method (ISM) is quite cost-effective as it eliminates the need for any laboratory equipments employed in media preparation. Furthermore, the obtained correlation coefficient within the (-0.85 to -1.0) and the coefficient of determination (R^2) more than 0.75, all are indicative of a high correlation between the Impedance and the reference method. Such findings, also confirmed by previous researches, form the basis for the mathematical equations and its application in food sciences. Finally, as a result of high correlation between these two methods in the detection of mesophiles in mayonnaise, i.e., 88.4%, and 93.2% for the winter and summer samples, respectively; the impedance method is considered a suitable alternative in the quantitative detection of bacteria in mayonnaise.

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