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MICROBIOLOGICAL QUALITY ASSESSMENT OF MINCED MEAT – CONVENTIONAL OR ALTERNATIVE METHOD?

Summary. Applications of alternative analytical methods in microbiology are conditioned by time-saving and economical reasons, but primarily for better performance. This study was conducted to determine the correlation between the standard count plate method and the TEMPO® method for minced beef samples under routine laboratory conditions. Three quality control indicators were selected for the test: total mesophilic microflora (total viable count), coliforms and *Escherichia coli*. The reference method procedures were conducted in accordance with ISO standards. 48 minced beef samples, contaminated at the level of 10^0 to 10^7 CFU/g by microflora isolated from commercial meat samples (mesophilic microflora, coliforms) and *E. coli* ATCC 8739, were examined. Statistical analysis of the results reveals substantial correlation between the alternative method and the standard count plate method. A linear correlation was found for all the types of tests. Correlation coefficients were high: 0.9977 for total mesophilic microflora, 0.9968 for coliform count, and 0.9970 for *E. coli* count. This correlation between the two methods makes them equal in result quality.

Key words: minced meat, microbiological contamination, count plate method, TEMPO® method

Introduction

Due to its high protein content and neutral pH, meat constitutes excellent growth medium for microflora. After slaughter, numerous bacteria of the genera *Pseudomonas*, *Alcaligenes*, *Escherichia*, *Micrococcus*, *Streptococcus*, *Proteus*, *Bacillus* and *Clostridium* can be found on the carcass surfaces. Other abundant species include *Mucor*, *Rhizopus*, *Penicillium*, *Aspergillus*, and *Cladosporium* molds as well as *Candida*, *Rhodotorula*, *Saccharomyces* and *Torulopsis* yeasts. Apart from the prevalent saprophytes, also some pathogenic bacteria may occur, such as *Salmonella* sp. or *Yersinia* sp. (ZALESKI 1985). The microbiological purity of meat is one of the crucial determinants of its shelf life, and the amount of microflora on the carcass surface is considered to be a measure of slaughter hygiene. The determination of microbiological purity is particu-

larly important in the case of minced meat, as apart from the microflora brought with the carcass, additional microorganisms are introduced during meat grinding and packing. Prompt and reliable microbiological assessment of minced meat is vital both for producers and consumers, who expect to purchase fresh and safe commodities.

Microbiological requirements for carcass production hygiene stipulated in European Union standards include testing for total mesophilic microflora, *Enterobacteriaceae* and *Salmonella* sp. For ground meat and mechanically separated meat, testing for *Escherichia coli* count should also be done. These parameters, providing full information about the microbiological purity of products, are called Quality Indicators (QI) (BETTS 2005).

Performing plate counts or most probable number testing (MPN) is time-consuming, material-intensive and laborious. Decreasing the costs of microbiological services can be done through implementation of automatic systems, miniaturized tests, automatic readout, and computer analysis of results. Automated systems can examine a number of samples in a short time, while instrumental methods are now more and more widely used in quantitative analysis procedures designed to determine counts of particular categories of microorganisms. One of the devices in the Polish market is the TEMPO® system (bioMerieux), which offers, among others, the following options: TVC (Total Viable Count) – for mesophilic microflora enumeration, TC (Total Coliforms) – for coliform enumeration and EC (Escherichia coli) – for *Escherichia coli* enumeration.

The TEMPO® system consists of two independent units, TEMPO® Filler and TEMPO® Reader, which are connected to a computer control unit. The station for direct work with biological materials (sample preparation, inoculation, and card filling) and the reading station can be located in different rooms. Therefore, the reading station does not come in direct contact with any biological material. Media for inoculation are supplied in lyophilized form, in separate bottles for each sample. After medium rehydration and adding the appropriate dilution of the material tested, the suspension is automatically transferred to a plastic card with sterile wells, having three volumes each. The principle of estimation in the TEMPO® system depends on either generating or quenching a fluorescent signal as a result of the reaction of growth medium components with metabolites created during microbial growth. The system determines microbial count by detecting positive wells and then performs statistical analysis by the MPN method.

Alternative analytical methods in microbiology are introduced not only for time and cost saving reasons, but also primarily for better performance. This study was conducted to determine the correlation between the standard count plate method and the TEMPO® method for minced beef samples under routine laboratory conditions. Three quality control indicators were selected for the test: total mesophilic microflora (Total Viable Count), coliforms and *Escherichia coli*. Reference method procedures were conducted in accordance with ISO standards.

Material and methods

The study was performed with naturally contaminated minced beef purchased in a Polish market.

Microorganisms. Mesophilic microflora was isolated from the meat sample by non-selective cultivation in Trypticase Soy Broth (TSB, Oxoid, UK) at 30ºC for 72 h.

A group of coliforms inhabiting minced beef was selectively grown on Violet Red Bile Lactose Agar (VRBL, bioMerieux, Poland) at 30ºC for 48 h. *Escherichia coli* ATCC 8739 strain used in the study originated from the American Type Culture Collection. The stock cultures of bacteria were maintained on Trypticase Soy Agar slants (TSA, Oxoid, UK). Before each experiment, the microorganisms were activated through double passaging on TSA medium (Trypticase Soy Agar, Oxoid, UK): the mesophilic microflora and coliforms at 30ºC for 48 h and *Escherichia coli* at 37ºC for 48 h.

Sample preparation. 48 minced meat samples were sterilized by irradiation and their sterility was tested by count plate method (PCA medium, bioMerieux, Poland, 30ºC, 72 h).

Inocula of mesophilic microflora, coliforms and *Escherichia coli* were prepared in Buffered Peptone Water (BPW, bioMerieux, Poland) and adjusted to the final concentration of approximately 10^8 CFU/ml. Each 10 g minced beef sterile sample was contaminated by microorganisms to a defined level of microflora: from 1 to 10^7 CFU/g.

10 g meat samples, after simultaneous inoculation with mesophilic microflora, coliforms and *Escherichia coli*, were aseptically transferred to a TEMPO® bag with a filter and homogenized with 90 ml BPW in a stomacher-type blender. The solution from the filter compartment was taken for further examination. The same sample was used to compare the routine reference method and the alternative TEMPO® method.

Microorganism enumeration by ISO reference methods. The number of mesophilic microorganisms was evaluated using the plate count method on PCA medium at 30ºC in accordance with PN-ISO 4833:2004. The number of coliforms was established using the VRBL medium in accordance with PN-ISO 4832:2007. *Escherichia coli* was counted using the Coli ID medium (bioMerieux, Poland) in accordance with PN-ISO 16649-1:2004. The results were calculated as the average number of six parallel plates for each dilution.

Microorganism enumeration by alternative TEMPO® method. TEMPO® tests were performed in compliance with the procedure recommended by the manufacturer, bioMerieux. Every meat sample was tested by the TEMPO® method for TVC enumeration (Total Viable Count – mesophilic microflora), TC enumeration (Total Coliforms) and EC enumeration (Escherichia coli) at dilutions corresponding to the estimated levels of sample contamination. The TEMPO® dilution was set to correlate with the dilution used for the conventional reference method. The results were calculated as an average from six parallel tests for each dilution.

Statistical analysis. Results were presented as an arithmetic mean of 6 determinations and were analysed using a three-way ANOVA test at a confidence level of $p < 0.05$. The results obtained by the standard count plate method were compared with the TEMPO® results. The linear regression fit according to the formula $Y = A + B \times X$ at a confidence level of $p < 0.05$ was applied. Samples leading to a difference of over 1 log unit between TEMPO® and the reference method were considered discordant. The statistical analysis was performed by means of ORIGIN® 6.1 software.

Results and discussion

Assessment of correlation between the alternative method and the reference count plate method for minced beef was conducted in model conditions. However, in order to

simulate natural conditions, the sterile meat samples were contaminated with microflora isolated from commercial meat samples (Total Viable Count, Coliforms). As no *Escherichia coli* was found in the meat purchased, a collection reference strain was used in the tests. The reliability of TEMPO® testing was evaluated at contamination levels ranging from 10^0 to 10^7 CFU/g (Table 1).

Table 1. Comparison of microorganisms' enumeration in minced meat by count plate reference method and TEMPO® method
Tabela 1. Porównanie referencyjnej metody płytkowej i metody TEMPO® w określaniu liczby drobnoustrojów w mięsie mielonym

Type of enumeration	Cell numbers (log CFU/g)		Correlation coefficient
	reference method	TEMPO® method	
Total Viable Count	0.73 ±0.09	0.77 ±0.06	0.9977
	1.80 ±0.31	1.62 ±0.37	
	2.53 ±0.20	2.55 ±0.43	
	3.37 ±0.38	3.51 ±0.34	
	4.52 ±0.24	4.49 ±0.24	
	5.52 ±0.25	5.59 ±0.33	
	6.48 ±0.27	6.54 ±0.27	
	7.47 ±0.25	7.47 ±0.25	
Total Coliforms	0.51 ±0.18	0.56 ±0.15	0.9968
	1.57 ±0.37	1.37 ±0.29	
	2.54 ±0.20	2.50 ±0.20	
	3.54 ±0.27	3.33 ±0.26	
	4.43 ±0.26	4.46 ±0.33	
	5.45 ±0.27	5.27 ±0.17	
	6.45 ±0.28	6.38 ±0.17	
	7.40 ±0.25	7.39 ±0.26	
Escherichia coli	0.54 ±0.13	0.55 ±0.15	0.9970
	1.24 ±0.28	1.24 ±0.31	
	2.36 ±0.29	2.33 ±0.29	
	3.40 ±0.36	3.50 ±0.31	
	4.42 ±0.30	4.60 ±0.26	
	5.54 ±0.48	5.52 ±0.47	
	6.59 ±0.26	6.54 ±0.17	
	7.22 ±0.09	7.24 ±0.09	

The results are means of six assays followed by standard deviation.

For all the three test types (TVC, TC and EC), the results of six parallel assays per sample were found to be highly consistent. The standard deviation was 0.06 to 0.43 logarithmic units per gram for TVC TEMPO®, 0.15 to 0.33 logarithmic units per gram for TC TEMPO®, and 0.09 to 0.47 logarithmic units per gram for EC TEMPO®, so it did not exceed 0.47 logarithmic units per gram for any of the tests. Irrespective of test type, the microbial counts determined by the reference count plate method and the alternative method revealed considerable conformity. Differences in results between the methods do not exceed 0.5 logarithmic units per gram, which confirms the performance declared by the producer (under 1 logarithmic units per gram) (TEMPO®... 2006). Statistical analysis of the results indicates a high correlation between the alternative method and the count plate method. A linear correlation was found for all the types of assays. Correlation coefficients were high: from 0.9968 for coliform count to 0.9977 for mesophilic microflora count (Table 1). The distribution of measurement points for particular assays in linear regression at a confidence level of $p < 0.05$ is presented in Figures 1-3. The widely used ranking of results according to the correlation indexes (TAVOLARO et AL. 2005) classifies correlation as excellent with correlation coefficient of 1.00-0.90, slope 1.00-0.90, and intercept 0.00-0.10. These conditions were met for the three test types discussed (Figs. 1-3).

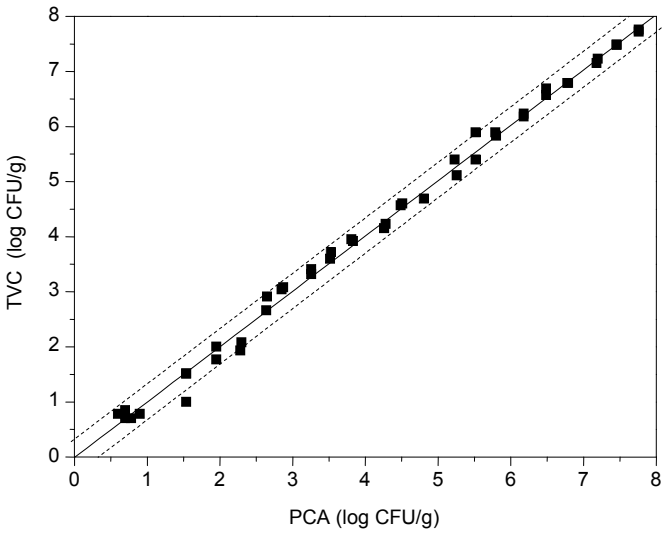

Fig. 1. Linear regression curve for mesophilic microflora enumeration in minced meat, $Y = -0.0622 + 1.00658 \times X$, $r = 0.9977$, confidence level $p < 0.05$; TVC – cells' number by TVC TEMPO® method, PCA – cells' number by count plate method on PCA medium

Rys. 1. Regresja liniowa dla określenia liczby drobnoustrojów mezofilnych w mięsie mielonym, $Y = -0{,}0622 + 1{,}00658 \times X$, $r = 0{,}9977$, przedział ufności $p < 0{,}05$; TVC – liczba komórek wyznaczona metodą TVC TEMPO®, PCA – liczba komórek wyznaczona metodą płytkową na pożywce PCA

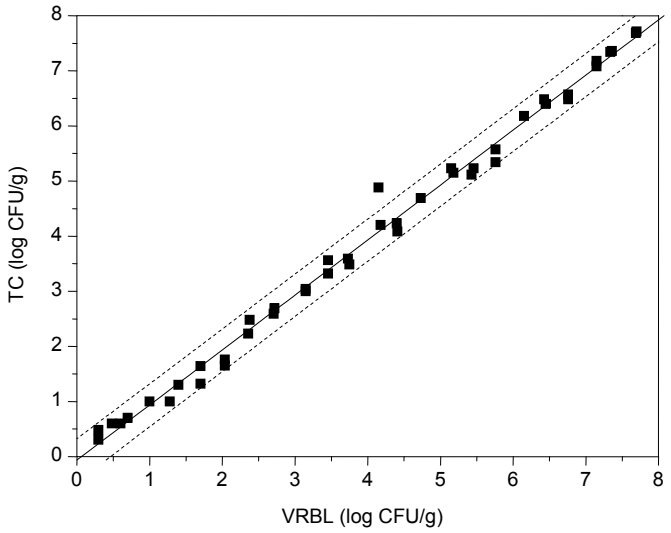

Fig. 2. Linear regression curve for coliforms enumeration in minced meat, Y = –0.06897 + 0.99868 × X, r = 0.9968, confidence level $p < 0.05$; TC – cells' number by TC TEMPO® method, VRBL – cells' number by count plate method on VRBL medium
Rys. 2. Regresja liniowa dla określenia liczby bakterii z grupy coli w mięsie mielonym, Y = –0,06897 + 0,99868 × X, r = 0,9968, przedział ufności $p < 0,05$; TC – liczba komórek wyznaczona metodą TC TEMPO®, VRBL – liczba komórek wyznaczona metodą płytkową na pożywce VRBL

Minced beef constitutes a food matrix that is often used in comparative studies of microbiological analysis methods (KUNICKA 2007, RUSSEL 2000, 2001). For automatic systems based on optical (bioSys® BioSys, Inc.) or conductometric (Bactometer®, bioMerieux) culture detection, the coefficients of correlation with standard methods of conducting *Escherichia coli* and coliform counts in ground beef ranged from 0.93 to 0.96 (RUSSEL 2000, 2001). Other methods based on fluorimetric culture testing, similarly to the TEMPO® method, ensure a high degree of correlation with the standard methods for Quality Indicators parameters in ground beef (correlation coefficients of 0.83-0.94) (KANG et AL. 2003, OH et AL. 2004). Previous examinations of the TEMPO® system for different food matrices in terms of total viable count, coliform count and *Escherichia coli* count also confirm a high correlation with the reference methods (0.95--0.99) (KUNICKA 2007).

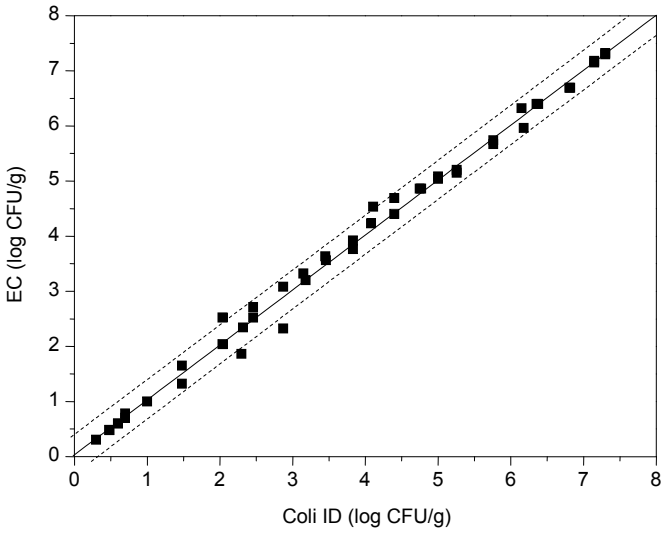

Fig. 3. Linear regression curve for *Escherichia coli* enumeration in minced meat, Y = 0.04241 + 0.99593 × X, r = 0.9970, confidence level p < 0.05; EC – cells' number by EC TEMPO® method, Coli ID – cells' number by count plate method on Coli ID medium
Rys. 3. Regresja liniowa dla określenia liczby bakterii *Escherichia coli* w mięsie mielonym, Y = 0,04241 + 0.99593 × X, r = 0,9970, przedział ufności p < 0,05; EC – liczba komórek wyznaczona metodą EC TEMPO®, Coli ID – liczba komórek wyznaczona metodą płytkową na pożywce Coli ID

Conclusions

The study reveals a high correlation between the alternative method of conducting total mesophilic microflora counts, coliform counts and *Escherichia coli* counts in minced beef and the reference count plate method in accordance with ISO procedures. This correlation between the two methods makes them equal in result quality. The alternative method offers simplified testing procedures, accelerating tests' numbers and cutting costs of analysis.

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OCENA JAKOŚCI MIKROBIOLOGICZNEJ MIĘSA MIELONEGO – METODA KONWENCJONALNA CZY ALTERNATYWNA?

Streszczenie. Zastosowanie alternatywnej metody badań w analizie mikrobiologicznej jest uwarunkowane nie tylko skróceniem czasu analizy i efektem ekonomicznym, a przede wszystkim jej wiarygodnością. Celem przeprowadzonych badań było określenie korelacji pomiędzy standardową metodą płytkową a metodą TEMPO® dla próbek mielonego mięsa wołowego, w warunkach laboratoryjnych. Do testu wybrano trzy wskaźniki kontroli jakości: ogólną liczbę drobnoustrojów mezofilnych, bakterie z grupy coli i bakterie *Escherichia coli*. Procedury metod referencyjnych przeprowadzono według standardów ISO. Wykonano badania 48 próbek mielonego mięsa wołowego zanieczyszczonych na poziomie od 10^0 do 10^7 jtk/g. Statystyczna ocena wyników wskazuje na dużą korelację metody alternatywnej ze standardową metodą płytkową. Stwierdzono korelację liniową dla wszystkich rodzajów oznaczeń. Współczynniki korelacji przyjmowały dużą wartość: 0,9977 dla oznaczenia liczby drobnoustrojów mezofilnych, 0,9968 dla oznaczenia liczby bakterii z grupy coli i 0,9970 dla *Escherichia coli*. Duża zgodność wyników uzyskanych w obu metodach wskazuje na równocenność metody referencyjnej i niekonwencjonalnej, co umożliwia automatyzację rutynowych badań mikrobiologicznych mięsa mielonego.

Słowa kluczowe: mięso mielone, zanieczyszczenie mikrobiologiczne, metoda płytkowa, metoda TEMPO®

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