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TEMPO® SYSTEM IN MICROBIOLOGICAL ANALYSIS OF SPICES AND FOOD ADDITIVES

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Key words: microbiological analysis of spices, Most Probable Number (MPN) method, instrumental microbiological analysis

Thanks to the research it was possible to evaluate how useful is the automated TEMPO® system (bioMerieux) for determining the total number of mesophilic microorganisms in spices and food additives, characterised by a high concentration of dyes. Statistical analysis of the results by linear regression proves the equivalence of the TEMPO® method and the standard count plate method, at a correlation coefficient of 0.99. According to the findings, the dye of the examined matrices does not influence the result of the fluorescent signal at sample dilution of 1/4 000, which results in microbiological matrices contamination, not lower than 1.0×10^3 CFU/g. In the case of colourful food matrices demonstrating a high level of contamination with mesophilic microflora, such as whole black peppercorns (the level of microflora above 4.9×10^6 CFU/g), sample dilutions of 1/40 000 should be applied.

INTRODUCTION

Over the last decade there has been considerable progress in instrumental microbiological analysis of food. Thanks to the development of numerous automated systems it is possible to examine a lot of samples within a relatively short period of time. Instrumental methods are widely used in quantitative analysis, which gives the number of microorganisms that belong to a certain group [Betts, 2000; Russel, 2001]. The instrumental method used in microbiological analysis should be reliable and take less time comparing to the standard method. It is vital to decrease the amount of labour work, the costs and the biological waste produced in the course of such an analysis. Taking into account the necessity of conducting a large number of tests, it is important to simplify the analytical procedure, make any units operations as automated as possible and introduce instruments to the system which would be easy to operate. The reliability of the instrumental method is estimated by the correlation with the standard method for a particular food matrix [Russel, 2000; Kunicka, 2006]. The TEMPO® system (bioMerieux) is designed to control microbiological Quality Indicators. Quality Indicators such as the total number of mesophilic microorganisms, the number of coliforms and *Escherichia coli* or, finally the number of yeasts or moulds give information on the state of the product or the environment [Betts, 2005]. The system is addressed for food producers where raw food, semi-processed products and final products as well as the production environment are monitored and checked to trace any microbiological contamination. The producer usually establishes the number and kinds of tests in such a way to ensure the final product safety.

The TEMPO® system consists of two independent devices: the TEMPO® Filler and the TEMPO® Reader, which are connected to the main computer. Separating the Filler from the Reader and wireless computer network allows preparation stations to be located in separate rooms (preparing samples, inoculation, card filling) and reading stations, located far from the analysed biological material. Culture media used for inoculation are lyophilized and provided in separate bottles for each sample. After rehydration of the medium and introducing proper dilution of the analysed material, the suspension is automatically placed in a plastic card, which contains three sets of sterile wells in three volumes with one logarithmic unit difference. Depending on the test is being carried out, the fluorescent signal is either generated or becomes extinct, which results from the reaction of the medium components with the metabolites produced during the growth of microorganisms. The TEMPO® system gives the number of microorganisms by reading positive wells and then performs a statistical analysis with the use of Most Probable Number (MPN) method.

The fluorescent signal can be distorted in the case of some substances conveying intensive dyes, such as herbal seasonings, vegetable concentrates or animal origin extracts, because these dyes might interfere with the signal generated by the instrument. In such situations one should verify whether the analysis performed with the use of the TEMPO® system is reliable as well find the smallest dilution of the sample that would not distort the findings of the reading. In the experiment, food matrices with different intensification of dyes, starting with lyophilized onion grits or yeast extract and finishing with mushroom stock cubes and lyophilized juice concentrate made from beetroot, were used to determine the level of mesophilic microflora of nine matrices, which were spices or food additives.

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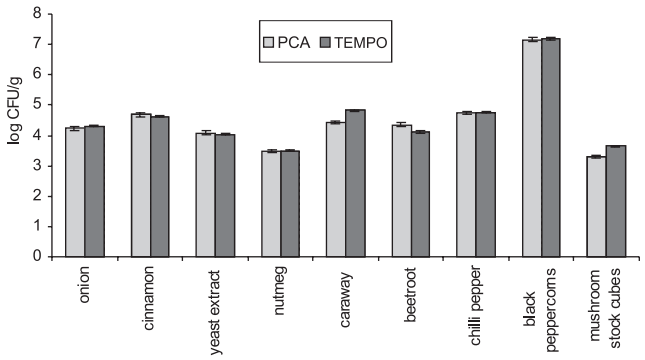

FIGURE 1. Spices microbiological contamination determined by standard count plate method (PCA) and the TEMPO® system.

MATERIALS AND METHODS

Lyophilized onion grits, cinnamon, yeast extract, nutmeg, ground caraway, juice concentrate made from beetroot, chilli pepper and mushroom stock cubes as well as dried black peppercorns were used in the analysis.

Preparation of the sample: 10 g of product was placed in 90 mL of buffered peptone water and then homogenized in a stomacher bag with a lateral filter; a filtrate was taken to perform further dilutions in buffered peptone water.

The total number of mesophilic microorganisms was determined with the use of both the methods: the TEMPO® method and the standard count plate method. The TEMPO® TVC test was carried out according to the manufacturer's recommendations, applying three dilutions of the analysed matrix, *i.e.* 1/400, 1/4 000 and 1/40 000. In the standard reference method PCA (bioMerieux) medium was applied and the analysis was performed in compliance with Polish Standard [PN-ISO 4833].

The results obtained after application of the standard count plate method and the TEMPO® method were analysed statistically by linear regression and with the use of computer programme ORIGIN® 6.1. The linear regression can be described by the following formula: Y=A + B x X, at a confidence level 95%. The results make up arithmetic mean of three reading findings. The standard deviation did not exceed 0.06 of logarithmic unit.

RESULTS AND DISCUSSION

Spices used in households by individual consumers as well as those used in food processing industry serve an important source of mesophilic microorganisms [Garcia *et al.*, 2001; Banerjee & Sarkar, 2003]. According to the ICMSF recommendations, the acceptable contamination level of the spices caused by the presence of aerobic mesophilic microorganisms cannot exceed 10^6 CFU/g. In the case of a good quality product, the contamination level should be lower than 10^4 CFU/g [ICMFS, 1974]. The results obtained after application of the standard count plate method prove that ground and lyophilized spices contained the amount of mesophylic microorganisms equal to $2.0 \times 10^3 - 5.4 \times 10^4$ CFU/g (Figure 1), which was in compliance with ICMFA standards. Out of all the analysed products the most contaminated was black peppercorn. Here the number of mesophilic microorganisms was higher by more than three logarithmic units comparing with the other products. Similarly, such high contamination with mesophilic microflora was seen in black peppercorns coming from Mexican [Garcia *et al.*, 2001] and Indian markets [Banerjee & Sarkar, 2003].

The problem that appears while applying the instrumental method for analysing spices, is a high concentration of the dye introduced along with the sample. In the TEMPO® system, there might be reading findings for four different dilutions of the sample, *i.e.* 1/40, 1/400, 1/4 000 and 1/40 000. The reading finding range depends on the dilution. At the application of the lowest dilution, the instrument will demonstrate the microbiological contamination ranging from 10 to 49 000 CFU/g. The working range of the TEMPO® system for higher dilutions was given in Table 1. Manufacturer's recommendations concerning the sample dilution, not lower than 1/400, might still lead to interference of the dyes and fluorescent signal. In the case of higher dilutions, the reading finding range might not cover the contamination of the sample. While application the TEMPO® system for the samples dilution 1/400, in case

TABLE 1. The results of microbiological contamination for different sample dilutions conducted by the alternative TEMPO® method.

Matrices / Dilutions range	Contamination level (CFU/g)		
	1/400	1/4 000	1/40 000
	$1.0x10^2 - 4.9x10^5$ CFU/g	$1.0x10^3 - 4.9x10^6$ CFU/g	$1.0x10^4 - 4.9x10^7$ CFU/g
Onion grits		$2.0x10^4$	$2.1x10^4$
Cinnamon	sample interference	$4.2x10^4$	$5.2x10^4$
Yeast extract		$1.1x10^4$	$1.0x10^4$
Nutmeg	$3.2x10^3$	$3.3x10^3$	sample out of range
Ground caraway	sample interference	$6.6x10^4$	$2.1x10^4$
Beetroot juice concentrate		$1.3x10^4$	$2.7x10^4$
Chilli pepper	$4.9x10^4$	$5.6x10^4$	$5.6x10^4$
Black peppercorns	sample out of range	sample out of range	$1.5x10^7$
Mushroom stock cubes	$6.8x10^3$	$4.4x10^3$	sample out of range

of five, out of nine analysed spices (onion grits, cinnamon, yeast extract, ground caraway and juice concentrate made from beetroot) interference of the sample with the reading signal was observed. The dilution shift up to 1/4 000 made it possible to obtain information on the level of mesophilic microflora of the analysed matrices, except for black peppercorns (Table 1). Because of a high level of microbiological contamination of the black peppercorns, the dilution of the sample should be 1/40 000. It was impossible to obtain right reading findings for the highest recommended dilution in case of the samples of nutmeg and mushroom stock cubes, since the level of microflora was lower than the range of work of the TEMPO® system (Table 1).

Regardless of the sample dilution applied, a high compatibility of the standard count plate method and the automated TEMPO® method was observed. Statistical analysis of the results by linear regression proves a good correlation of these two methods. The regression formula is the following: $Y = 4.8 \times 10^3 + 0.88\ X$ and concordance coefficient R is 0.99. Moreover, the differences in results between these two methods do not exceed 0.5 of the logarithmic unit (Diagram). Literature data prove the compatibility of the count plate method and the TEMPO® method. The base for that proof was the analysis of the mesophilic microflora in 145 samples of meat, poultry and egg products (linear correlation coefficient R = 0.93) [Betts, 2005]. The compatibility of the manual and instrumental methods was also observed in cases of food matrices such as: pork/beef mince (R = 0.98), cottage cheese (R = 0.97), frozen vegetables (R = 0.99) [Kunicka, 2006].

CONCLUSIONS

The analysis of food spices, characterised by high concentration of dyes, carried out with the use of the instrumental TEMPO® method was proved to be compatible with the standard count plate method. The TEMPO® system can be used to analyse dyed products provided the level of microbiological contamination of the matrix is not lower than 1.0×10^3 CFU/g and the sample dilution 1/4 000.

In the case of matrices highly contaminated by the mesophilic microflora, such as black peppercorns, with the level of microflora higher than 4.9×10^6 CFU/g, sample dilutions of 1/40 000 should be applied.

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SYSTEM TEMPO® W ANALIZIE MIKROBIOLOGICZNEJ PRZYPRAW I DODATKÓW DO ŻYWNOŚCI

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Dokonano oceny przydatności automatycznego systemu TEMPO® (bioMerieux) do określania ogólnej liczby drobnoustrojów mezofilnych w przyprawach i dodatkach do żywności o wysokim stężeniu barwników. Statystyczna ocena wyników za pomocą regresji liniowej wskazuje na dobrą zgodność metody TEMPO® ze standardową metodą płytkową (rys. 1), przy współczynniku korelacji równym 0,99. Nie stwierdzono wpływu barwników badanych matryc na odczyt sygnału fluorescencyjnego przy rozcieńczeniu próbki 1/4 000; co warunkuje poziom zanieczyszczenia mikrobiologicznego przypraw nie niższy niż $1{,}0 \times 10^3$ CFU/g (tab. 1). Dla barwnych matryc o wysokim zanieczyszczeniu mikroflorą mezofilną, takich jak pieprz czarny ziarnisty, wykazujących poziom mikroflory powyżej $4{,}9 \times 10^6$ CFU/g, należy stosować rozcieńczenia próbki równe 1/40 000.