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Evaluation of two rapid methods for enumeration of yeast and mould in food

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RESEARCH PAPER

Abstract

Several rapid methods have been developed for the enumeration of yeast and mould in foods. Among these rapid methods, the NEO-GRID hydrophobic grid membrane filter (HGMF) requires a considerably short incubation period and the TEMPO YM offers automation. TEMPO YM and NEO-GRID HGMF were compared with the current Food and Drug Administration (FDA) method, the reference method, for the enumeration of yeast and mould in foods. This study of method comparison was conducted using 261 naturally contaminated food samples including dried fruits, marmalade, flour, pastry, sausage, nuts, sauce and spices. The results of the statistical analysis of different food categories demonstrated that differences between the rapid methods and the FDA method were not significant (P>0.05). Both TEMPO YM and NEO-GRID HGMF are rapid and efficient alternative methods for the enumeration of yeast and mould in foods.

Keywords: BAM method, NEO-GRID HGMF, TEMPO YM, yeast and mould enumeration

1. Introduction

Yeasts and moulds are significant microorganisms that cause foods to spoil. Moulds are especially important for the food industry, because (a) they can grow in extreme conditions where many bacteria cannot grow; and (b) many strains of them produce mycotoxins, some of which are carcinogenic or mutagenic and may cause organ specific pathology such as hepatotoxic (Alldrick *et al.*, 2009; Ray and Bhunia, 2008). Therefore, an elevated level of mould often indicates a possible presence of mycotoxins in foods as well. For this reason, the level of fungal contamination in foods should be monitored (Tournas *et al.*, 2011).

Current conventional culture methods for enumeration of yeast and mould in foods require an incubation period of 5 days (FDA, 2001; ISO, 2008a,b). However, a rapidly available analysis result is a necessity to reduce the reaction time for a possible microbiological food safety risks. In addition, storage costs are substantially increased as the analysis durations increases (Kawasaki *et al.*, 2003). There has been an increasing demand for development of time-saving methods for enumeration of yeast and mould in foods. As a result of this demand, several rapid methods have been developed and introduced into the market for enumerating yeast and mould in foods.

Among recently developed rapid methodologies for yeast and mould count in foods, the TEMPO system (bioMérieux, Marcyl l'Etoile, France) has attracted remarkable attention due to its automated functions. The TEMPO system, based on traditional microbiology, is an automated most probable number (MPN) method. TEMPO YM (yeasts/moulds) is intended for exclusive use with the TEMPO system for the enumeration of yeasts and moulds in 72-76 hours in food products. The TEMPO YM test consists of a card and a vial of specific culture medium. The inoculated medium with food sample suspension is transferred by the TEMPO Filler into the card. During incubation, yeasts and moulds present in the card reduce the substrate in the culture medium and cause a fluorescent signal which is detected by the TEMPO Reader. Depending on the number and type of the fluorescent signal positive wells, results are calculated automatically by the TEMPO Reader (Anonymous, 2010).

The hydrophobic grid membrane filter (HGMF) method, one of the earliest rapid methods in quantitative microbiology, was first introduced to food microbiology by Brodsky *et al.* (1982). HGMF is a membrane filter imprinted with hydrophobic grids to form 1,600 individual growth squares. The hydrophobic grids restrict the lateral spread of colonies (Wu and Fung, 2004). Currently, two systems based on the HGMF method are commercially available for microbiological analysis of foods: ISO-GRID and NEO-GRID (Neogen, Lansing, MI, USA). The only difference between the two systems is use of disposable filter units, which are supplied with the NEO-GRID system.

The YM-11 agar is used in the NEO-GRID HGMF method for enumeration of yeast and mould in foods. YM-11 agar was first developed and optimised by Entis and Lerner (1996) for recovery of both yeasts and moulds on the HGMF filter within 48-52 hours. This medium contains trypan blue which is a dye that stains both yeasts and moulds (Tournas, 2009).

To date, there are limited published studies indented for evaluation of rapid methods for enumeration of yeast and mould in foods. The aim of this study is to evaluate the TEMPO YM and the NEO-GRID HGMF for the enumeration of yeast and mould in naturally contaminated food samples and compare them with the conventional culture method.

2. Materials and methods

Samples

In this study, a total of 261 naturally contaminated food samples were used. Yeast and mould counts of samples were determined as in range data by the three method protocols described below. The samples included dried fruits (23), marmalade (24), flour (29), pastry (33), sausage (36), nuts (36), sauce (37) and spices (43), which were obtained from different producers and retail outlets in Turkey.

Sample preparation

Ten grams of samples were homogenised with 90 ml 0.1% peptone water in the filter bag (bioMérieux) using a stomacher (IUL, Barcelona, Spain). Additional ten-fold dilutions were made with 0.1% peptone water. For each method, analyses were started from suitable dilution of samples depending on the methodology in order to obtain similar enumeration ranges among the methods.

FDA method

A conventional spread plate method was conducted according to the Food and Drug Administration (FDA) bacteriological analytical method (FDA, 2001). Depending on the water activity of the samples, an amount of 0.1 ml of initial suspensions and 1/100 dilutions were spread plated on Dichloran rose bengal chloramphenicol (DRBC) agar (Merck, Darmstadt, Germany) or DG18 agar (Lab M, Bury, UK) supplemented with glycerol. After incubation at 25 °C for 5 days, yeast and mould colonies on the plates were counted and results were calculated using an appropriate dilution factor. The enumeration range of the FDA method was from 100 to 1.5×10^5 cfu/g.

TEMPO YM method

One millilitre of the 1/100 dilutions of samples were transferred to TEMPO YM medium vials which were previously reconstituted with 3 ml of sterile distilled water. The TEMPO Filler automatically transferred these suspensions to the card that contains 48 wells with three sizes (16×225 µl, 16×22.5 µl, 16×2.25 µl). Since the total volume of the card is larger than the total volume of the diluted sample, gaseous headspaces were formed in each filled well. These headspaces ensured that the contents of the wells were kept separate from each other. As soon as the wells were filled, the transfer tube of the cards was hermetically cut and the cards were sealed. After 72 hours of incubation at 25 °C, readings were taken by the TEMPO Reader. The number of positive wells obtained, in relation to the volume of the wells and the dilution of the samples, provided the enumeration results in cfu per gram for the original samples, using the MPN tables (Anonymous, 2010). The enumeration range of one TEMPO card was from 100 to 4.9×10^5 cfu/g.

NEO-GRID HGMF method

One millilitre of the 1/100 dilutions of samples were added to NEO-GRID filter units containing Butterfield's phosphate buffer (prepared according to FDA, 2001) or Butterfield's phosphate buffer with 1% Tween 80 and vacuum-filtered through to a HGMF filter (Neogen). After the filtration, HGMF filters were removed from the filter units and placed on YM-11 agar (Acumedia, Lansing, MI, USA) supplemented with chlortetracycline-HCL. Plates were incubated at 25 °C for 50 hours. After the incubation, blue/greyish or blue coloured positive squares of filters were counted and these counts were converted to MPN values using a HGMF conversion table (CFIA, 1993). The enumeration range of the NEO-GRID HGMF method was 100 to 9.3×10^5 MPN/g.

Statistical analysis

Results were firstly converted to the logarithmic form. Then correlation coefficient and linear regression analyses were performed. Additionally, paired t-test was conducted to compare the mean values obtained from the rapid methods and the reference method. All statistical analyses were

performed using MiniTab statistical software (version 12.1; Minitab Inc., State College, PA, USA).

3. Results and discussion

In this study, two methods, TEMPO YM and NEO-GRID HGMF were compared to the current FDA method regarding enumeration of yeast and mould in naturally contaminated food samples including dried fruits, marmalade, flour, pastry, sausage, nuts, sauce and spices. The correlation coefficients between the rapid methods and the FDA method were higher than 0.90 for overall samples. This value is regarded as excellent in method comparison studies (Ferrati *et al*., 2005). The *P*-values obtained from the paired t-test showed that differences between the rapid methods and FDA method were not significant ($P>0.05$) for all food categories.

Correlation coefficients between the results of TEMPO YM and FDA method for different sample categories were calculated as between 0.822 and 0.942 (Table 1). For overall samples, a correlation coefficient of 0.910 was determined between the two methods. Regression analysis yielded the following equation: $\log_{10}$(TEMPO YM) = 0.466 + 0.876×BAM method (Supplementary Figure S1). Paired t-test yielded *P*-values ranging from 0.299 to 0.790 (Table 1). Similar results were obtained from a recent study of Katase and Tsumura (2011), who reported a high correlation (r>0.97) between the TEMPO YM and potato dextrose agar in artificially contaminated soy products. In another recent study, carried out by Lakićević *et al*. (2011), a high correlation (r>0.93) was reported between TEMPO YM and DG18 agar in both naturally and artificially contaminated food samples included dietetic products, seafood, spices and dairy products.

TEMPO YM has a limitation for highly coloured samples such as cocoa and fruit puree because the fluorescent signal may be affected if the primary dilution is strongly coloured (Anonymous, 2010). Owen *et al*. (2010) reported that false positive results or abnormal counts may be obtained with the TEMPO system methods from the initial suspension of highly coloured food samples. They also suggested that use of more diluted samples can overcome this difficulty. In this study, we used further decimal dilution of samples and did not observe any outlier count probably resulted from the colour interference.

TEMPO YM offers automated reading and recording of results, and substantial cost savings in terms of labour, by eliminating the need for serial dilution. Moreover, the TEMPO barcode system ensures complete traceability, thereby standardising the method. On the other hand, the major disadvantage of the TEMPO YM is the cost of the expensive specific TEMPO Filler and TEMPO Reader devices. Additionally, routine maintenance cost of the TEMPO System is also substantial. However, these cost disadvantages can be minimised by using the TEMPO system multiple for the additional microbiological quality indicators testing of foods. Therefore, it can be said that TEMPO System is more suitable for large-scale food microbiology laboratories.

Design of TEMPO cards offers additional advantages to TEMPO YM method. In the plate count technique, precision decreases as the number of colonies decreases (ISO, 2007). The number of miniaturised tubes in the TEMPO card increases enumeration range and the precision of results compared to a traditional MPN technique (Torlak *et al*., 2008). Zitz *et al*. (2011) reported that miniaturised tube design (3×16 tubes) of the TEMPO system significantly

Table 1. Mean total yeast and mould counts using the FDA, TEMPO YM or NEO-GRID HGMF method ($\log_{10}$ cfu/g), correlation coefficients (r) and *P*-values yielded by the paired t-test.

Food categories	No. of samples	Mean counts			FDA vs. TEMPO[1]		FDA vs. NEO-GRID[2]	
		FDA	TEMPO YM	NEO-GRID HGMF	r	P	r	P
Dried fruits	23	3.29±0.61	3.32±0.51	3.31±0.59	0.822	0.660	0.931	0.600
Marmalade	24	3.72±0.55	3.76±0.59	3.81±0.58	0.937	0.299	0.870	0.136
Flour	29	3.64±0.82	3.69±0.83	3.67±0.76	0.942	0.335	0.946	0.553
Pastry	33	3.89±0.61	3.86±0.64	3.96±0.51	0.908	0.433	0.894	0.155
Sausage	36	3.41±0.77	3.45±0.66	3.45±0.64	0.890	0.477	0.840	0.537
Nuts	36	3.38±0.67	3.40±0.68	3.32±0.62	0.881	0.790	0.815	0.317
Sauce	37	4.00±0.86	3.98±0.80	4.03±0.75	0.912	0.761	0.933	0.480
Spices	43	4.12±0.85	4.08±0.86	4.11±0.75	0.880	0.418	0.904	0.946
Total	261	3.72±0.79	3.72±0.76	3.74±0.73	0.910	0.811	0.902	0.244

[1] FDA compared with TEMPO YM.
[2] FDA compared with NEO-GRID HGMF system.

improved the precision of results compared to the traditional three tubes design of the MPN technique.

Correlations coefficients between the results of the NEO-GRID HGMF and FDA method for different sample categories were calculated between as 0.815 and 0.946 (Table 1). For overall samples, linear regression analysis gave a correlation coefficient of 0.902, and regression analysis yielded the following equation: $\log_{10}$(NEO-GRID HGMF) = 0.635 + 0.835×BAM method (Supplementary Figure S2). Paired t-test yielded *P*-values ranging from 0.136 to 0.946 (Table 1). This result is consistent with those of Tournas (2009), who reported a high correlation and no significant differences between the NEO-GRID HGMF method and FDA method in 244 naturally contaminated food samples. Previous studies on comparison of ISO-GRID HGMF method and reference culture methods for enumeration of yeast and mould in foods also showed that the combined use of HGMF technique and YM-11 agar is clearly preferable to the reference culture methods (Entis, 1996; Spangenberg and Ingham, 2000).

In NEO-GRID HGMF method, yeasts yielded blue colonies on filter, while moulds yielded greyish blue colonies. However, distinct coloration between yeast and mould colonies was not observed on some of the filters. Therefore, it should be noted that separate enumeration of yeast and mould in samples based on colour distinction of colonies by NEO-GRID HGMF method is not practical.

Considerably, short incubation time and large enumeration range obtained from single dilution are main advantages of NEO-GRID HGMF method compared to the FDA method. Nevertheless, NEO-GRID HGMF method requires laborious sample preparation and counting efforts. The NEO-GRID HGMF method may require enzyme treatment to improve the filtration of certain samples with thick initial suspension during sample preparation. Filter blockage is eliminated by digestion of proteins, starch, gum or cellulose in sample suspension. With NEO-GRID HGMF method, filtration can be effectively made without enzyme treatment when using further dilutions as inoculum instead of initial suspension (Tournas, 2009). Thus, use of 1/100 dilution of samples is more practical. The cost of initial set-up of membrane filter apparatus and vacuum pump, and cost of disposable filter units can be considered as another disadvantage of the NEO-GRID HGMF method.

The lack of significant differences and the excellent correlation between the results of two rapid methods and FDA method demonstrated that TEMPO YM and NEO-GRID HGMF are time-saving and reliable methods for the enumeration of yeast and mould in foods and can be alternate to the conventional methods.

In this study, as well as their performances, two rapid methods were also evaluated based on some method selection criteria such as cost. Objective of the analysis should also be considered when these methods are implemented in routine. The common disadvantage of the rapid methods evaluated in this study is that instead of individual counts of yeast and mould, a total count is obtained. Therefore, these methods can only be used when separate enumeration of yeast or mould and further identification is not required.

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Supplementary material

Supplementary material can be found online at http://dx.doi.org/10.3920/QAS2012.0113.

Figure S1. Results of linear regression of the FDA method ($\log_{10}$ cfu/g) vs. TEMPO YM ($\log_{10}$ cfu/g) in naturally contaminated food samples.

Figure S2. Results of linear regression of the FDA method ($\log_{10}$ cfu/g) vs. NEO-GRID HGMF ($\log_{10}$ cfu/g) in naturally contaminated food samples.

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