ESTIMATION OF MICROBIOLOGICAL QUALITY OF CHILLED AGED BEEF USING AUTOMATED TURBIDIMETRY

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Background.

Total Viable Count (TVC) determined by standard plate counts (SPC), as the reference methodology for enumeration of the total number of bacteria in foods, is generally regarded as the more common indicator of microbiological quality. However, the relatively long time to obtain the results (48-72 h) may be a disadvantage, especially in routine quality control. Various instruments developed to shorten the time of microbiological analysis of foods measure physical or chemical changes, i.e. optical density (OD), that are indicative of microbial growth. Turbidimetric methods are based on the fact that diluted bacterial suspensions absorb incident light proportionally to their concentration, according to Beer's law. Absorbance curves are obtained as the OD of the media increases after the number of bacteria has reached a threshold of detection. This threshold value is generally attained at high bacterial concentrations (approximately 10^7 CFU/ml), and involves a significant change of the OD. The time to reach the threshold of detection is called the detection time (DT), and depends on the initial concentration and the specific growth rate of bacteria (Begot et al, 1996).

Objective.

The objective of the present study was to evaluate the efficacy of an automated turbidimetric system to estimate the total bacteria load on fresh and aged retail chilled beef.

Material and Methods

Sampling and enumeration in agar plates: beef samples were obtained from a commercial, centralized, distribution operation. From each sample, an area of 14.73 cm^2 and 3 mm depth was removed in an aseptic manner, placed in a sterile bag containing 50ml of sterile 0.1% peptone water, and homogenized for 2 min in a stomacher (Lab Blender 400, Seward Med, England). From these homogenates, suitable decimal dilutions in 0.1 % peptone water were prepared. Enumeration of bacterial groups (TVC, *Enterobacteriaceae, Pseudomonadaceae, Lactobacillaceae*) and microorganisms (*Brochothrix thermosphacta*) in different selective media was carried out as in Lasta et al. (1995).

Description of the turbidimetric unit: The turbidimetric unit used was the Bioscreen C analyzer system (Labsystem, Finland). The system consists of an analyzer unit, which includes a dispenser/diluter, incubator/shaker, and measurement unit. The incubation temperature selection ranges between 1°C and 60°C with an accuracy of ±1°C. The main parameters given by the system are the background turbidity, the DT, the slope of the OD curve at the logarithmic growth phase, the maximum OD for the growth (MG), the turning point (TP), and the total area under the growth curve (A). DT can be considered an apparent lag phase, including the real lag phase plus the time to reach the threshold OD value after growth has started.

Calibration curves: to establish the microbiological quality of a given type of food by the turbidimetric method, the system has to be first calibrated against the SPC reference method, as calibration curves are product-specific. In accordance several samples of the product, ideally ranging from low to high levels of contamination, must be analyzed simultaneously by the two methods. Calibration curves are constructed by correlating one or several OD curve parameters to the log CFU values. In this study calibration curves were created from beef samples with a wide range of microbial loads. Seventy samples of retail beef packaged in an oxygen-permeable film were considered: 40 were obtained 48 to 72 h after slaughter (fresh beef) and the remaining 30 were from vacuum-packaged wholesale cuts aged for 7-45 days. Fresh and aged samples were held at 4 ± 0.5 °C for 72 h to simulate retail storage conditions before the analysis for each sample. Enumeration in the different agar media and the turbidimetric measurement in a non-selective medium were simultaneously performed. Turbidimetric measurements were done in triplicate by transferring 50 µl of sample into honeycomb wells containing 250 µl of tryptone soy broth (TSB). The increase in turbidity was monitored automatically every 30 min at wide band (420-580 nm) at 25°C for 24 hours. TP, MG, A and DT parameters from the OD curve were correlated to TVC values as CFU/cm^2 (dilution factor: 50-ml/14.73 cm^2).

Statistical analysis: regression analyses for fresh and aged samples were done using, a linear regression program (SAS, 1998). In order to determine if the two calibration lines for fresh beef (n_1 points) and aged beef (n_2 points) were significantly different an F statistic with 2 and (n_1+n_2–4) degrees of freedom was calculated as

$$F_{(2,n1+n2-4)} = \frac{(RSS_{1\,line} - RSS_{2\,lines}) / 2}{s^2}$$

where RSS_{1ine} is the Residual Sum of Squares for the one-line calibration model, and RSS_{2lines} and s^2 are the Residual Sum of Squares and estimated variance for the two-lines calibration model respectively.

Performance of the calibration curve: to estimate the performance of the calibration equation an independent set of 19 aged beef samples consisting of different commercial cuts (short plate 4 ribs bone-in, part of "pony" 6 ribs boneless, and *supraspinatum* muscle) aged for 15 to 45 days were used. As previously described samples were simultaneously analyzed by turbidimetry and plate counts. TVC values were compared with the confidence interval for the estimated bacterial loads.

Results and Discussion

Enumeration in agar: Table 1 shows a summary of the composition of the flora in the samples used to create the calibration curves. It must be noted here the somehow unusual high counts in STAA and MRS agar for some of the aerobically stored samples.

Calibration curves: from regression analysis for the individual parameters of the OD curve it was found that DT was the best predictor to estimate the microbial load in fresh or aged beef samples (Table 2).

log CFU/cm²

Figure 1 shows the calibration curves (log CFU/cm^2 *vs* DT) for aged (R^2= 0.89) and fresh beef samples (R^2= 0.75). The result of the Ftest ($F_{(2,66)}$ = 2.98) showed that the two calibration lines for fresh and aged beef were not significantly different at a 95% confidence level. Therefore, for the samples tested, a one-line model seems to be appropriate. This may reflect the fact that the composition of the flora from fresh and aged samples in these trials, was not as different as might be expected.

Performance of the calibration curve: after 7, 9, 10 and 17 days of refrigerated aging the hygienic and overall acceptability of beef samples were adequate, considering TVC and sensorial characteristics. However, the microbial count from samples aged for 45 days was higher than log 7 CFU/ml with undesirable sensorial changes (color, off-flavors) observed by the sensory panel (data not shown). From the new set of aged samples (n=19), mean values of TVC obtained by plate counts and by turbidimetric measurements were determined as log 5.92 ± 1.71 and 5.54 ± 1.28 CFU/cm^2 respectively. For the additional set of aged beef samples estimated bacterial loads from DT values were, with one exception, inside a 95% confidence limit for the mean predicted response (Fig. 2). This corroborates that the calibration equation could be appropriate within the tested range.

Traditional microbiological methods are time consuming, whereas rapid automated methods, like turbidimetry, can obtain results indicating the hygienic quality of various foods in much shorter periods of time. DT found in this study are similar to those reported by Cartier et al. (1994) regarding a contamination level of approximately 10^4 microorganism per g or ml. This number of bacteria was detected in this assay after a period of 11 to 12 h incubation at 25 °C. For samples with higher counts, a shorter DT was observed: 6 to 8 h for 10^6 CFU values and 4 to 6 h for counts of 10^7 CFU. The present study indicates the potential that an automated turbidimetric technique has for enumerating bacteria in meat samples. However for each particular type of food, the specific relationship between plate count values and turbidimetric data must be assessed.

Conclusion

The results of the current assay indicated that an automatic turbidimetric method, using a microbiological analyzer, can estimate bacterial load in chilled beef samples in a rapid and efficient way.

References.

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Table 1. Minimum-maximum counts (log CFU/cm^2) for the different bacterial groups in fresh and aged beef samples used in calibration.

	TVC	*Pseudomonaceae*	*B. thermosphacta*	*Lactobacillaceae*	*Enterobacteriaceae*
Fresh beef	3.30-8.51	1.93-8.03	2.00-8.12	1.93-7.64	0.23-4.36
Aged beef	1.93-7.70	1.93-5.55	1.93-7.70	1.93-6.95	0.23-5.15

Table 2. Statistical parameters from calibration curves (log CFU/cm^2 *vs* DT)

	Fresh samples	Aged samples	Total samples
N° Observations	40	30	70
Determination coefficient (R^2)	0.75	0.89	0.83
Standard error	0.62	0.47	0.58

Fig. 1. Calibration curves for fresh and aged samples

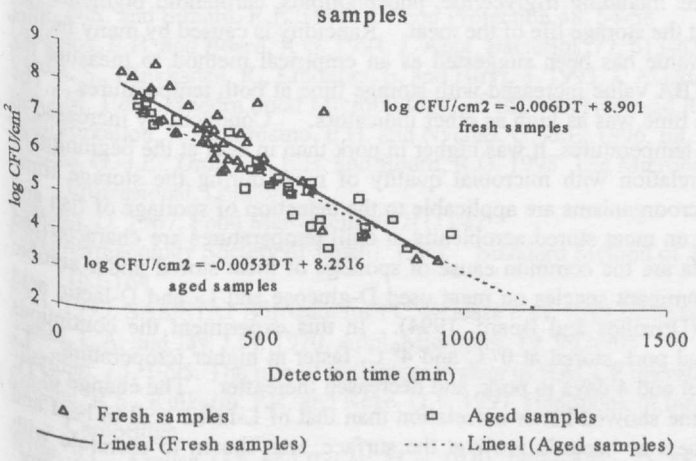

Fig. 2. Estimated bacterial load for aged beef samples