RAPID METHODS TO PREDICT BACTERIAL LOAD IN GROUND BEEF

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SUMMARY

Fluorescein diacetate hydrolysis (FDA), resazurin reduction time (RR), aminopeptidase activity (AP), absorbance at 600 nm (A_{600}), aerobic plate count (APC) and color ("a" value) of meat were monitored in refrigerated ground beef patties during storage. Analyses were also performed on spoiled, frozen patties to determine if freezing would alter the measured variable responses. Throughout the experiment, as bacterial load (APC) increased, FDA, AP, and A_{600} increased, while RR decreased. Regression analysis between APC and each of the other parameters revealed "r" square values of 0.88, 0.90, 0.86, 0.93, and 0.78, for FDA, RR, AP, A_{600}, and "a" value, respectively.

Introduction

Meat is a highly perishable product due to contamination by a variety of microorganisms. The growth of spoilage bacteria results in abbreviated shelf-life of meat, causing economic loss to the meat industry. Conventional methods for determination of total bacterial count are time-consuming. Practical application of many methods is limited by the requirement for expensive instrumentation and chemicals. Thus, it would be useful to have rapid, inexpensive and accurate methods for predicting the bacterial load of meat.

Fluorescein diacetate (FDA, 3',6'-diacetyl-fluorescein) is a synthetic substrate hydrolysed by a number of different enzymes such as proteases, lipases, and esterases (Guilbault and Kramer, 1964). The product of the enzymatic hydrolysis is fluorescein which can be quantified by spectrophotometry. FDA hydrolysis has been used to determine the amount of active microbial biomass in soil and needle litter (Swisher and Carole, 1980; Schnurer and Roswall, 1982), but not in food. Resazurin reduction (RR) has been used as an objective test of initial bacterial load in milk (A.P.H.A., 1967) and meat (Dodsworth and Kempton, 1992). The test measures the reducing activity of oxygen-consuming bacteria by recording the time required for the dye color to change (violet to pink to colorless) during incubation.

Aminopeptidase (AP) is an enzyme present in the cell walls of Gram-negative bacteria which can cleave L-alanine-p-nitroanilide to yield the chromophore p-nitroaniline. The activity of this enzyme has been reported to increase with bacterial load of whole meat cuts (Alvarado et al., 1992), but has not been reported for ground meats.

The population density of bacteria growing in liquid media is commonly assessed by determining the A_{600}. Mattila (1987) reported an automated turbidimetric method to predict the bacterial load in a variety of foods. The absorbance at 600 nm of the bacteria recovered from meat could be used as a rapid index of bacterial load, if background turbidity from meat did not interfere.

The bright red color of meat is due to the pigment oxymyoglobin and undesirable discoloration of meat occurs due to the accumulation of brown metmyoglobin. Bacterial contamination of meat appears to accelerate oxymyoglobin oxidation which results in undesirable color. Meat color can be quantitatively measured by colorimetry; high "a" values indicate more red color; as oxymyoglobin oxidizes, "a" values decrease. The hypothesis behind this approach is that as meat spoils, its "a" value decreases and this can be related to an increase in bacterial load.

Methods for predicting bacterial load should be applicable to both fresh and frozen meat. The present study was undertaken to examine if FDA hydrolysis, resazurin reduction test, aminopeptidase activity, absorbance at 600 nm and color of meat could be used as rapid indices of bacterial load in fresh and frozen ground beef.

Materials & Methods

The experiment was conducted on ground beef patties (n=5 per sampling day) over a 9 day storage period. The patties (thickness 0.6cm) were obtained from the U.S Army Natick Research, Development and Engineering Center, Natick, MA. All samples were packaged in oxygen permeable polyethylene film and kept frozen until the experiment was started. On day 0 (fresh baseline), five frozen samples were bored through transversely by means of a sterile meat corer (dia 3cm). Cores were each weighed (ca. 7.8 g) and transferred to a sterile stomacher bag containing 50 ml sterile peptone water (0.1%). The stomacher bag was then inverted twenty times and the solution used for determination of aerobic plate count, resazurin reduction, FDA hydrolysis, aminopeptidase activity and A_{600}. In addition, the "a" value on the outside surface of samples was also recorded. The remaining thirty-five samples were abused at 25°C for 24 hr, and after 24 hr the aforementioned measurements were again made on five samples. The remaining abused patties were then stored at 4°C and the parameters assayed on days 3, 5, 6, 7, and 8. After day 8, the remaining five samples packaged as before were refrozen at -38°C for 24 hr at which time all parameters were again measured to ascertain the effect of freezing at spoilage on assay responses.

Sample preparation: For resazurin reduction test, the diluent from the stomacher bag was directly used. For FDA hydrolysis, aminopeptidase activity, and A_{600}, 10 ml diluent (0.1% peptone solution containing meat homogenate) each was transferred into three sterile tubes and centrifuged (DPR-6000, International Equipment Co., MA) (170Xg for 30 sec) at 4°C to sediment meat particles. To confirm that centrifugation at this speed did not result in loss of bacteria, preliminary experiments were conducted by determining the bacterial count of the diluent before and after centrifugation at 170Xg for 30 sec. The supernatant was transferred into a new set of three tubes and centrifuged at 2000Xg for 30 min at 4°C.

Resazurin reduction test: Nine ml diluent from the stomacher bag was transferred into a sterile glass tube containing 1 ml resazurin reagent [11 mg resazurin (Sigma Chem. Co.) in 200 ml sterile distilled water]. The mixture was incubated at 25°C and the time required for each tube to change color from purple to pink was recorded (subjective determination).

FDA hydrolysis: The bacterial pellet from tube 1 was resuspended in 9 ml sterile sodium phosphate buffer (60 mM sodium phosphate dibasic, pH 7.6). The solution was then sonicated in a bath sonicator (Laboratory Supplies Co. Inc., NY) for 3 min and filtered (0.45 µm). To 3 ml of the resulting clear solution, 0.1 ml FDA reagent [500 µg FDA (Sigma Chem. Co.)/ml acetone] was added. The mixture was incubated at 25°C for 3 hrs and the absorbance of the solution at 490 nm was recorded (Swisher and Caroll, 1980) in a spectrophotometer (UV-2100, Shimadzu Corporation, Japan).

Aminopeptidase activity: The bacterial pellet from tube 2 was resuspended in 6 ml Tris buffer (pH 8) and the solution sonicated for 3 min and filtered (0.45 µm). To 2 ml of this solution, 0.25 ml 0.1% L-alanine-p-nitroanilide (Sigma Chem. Co.) was added. The mixture was incubated at 37°C for 2 hrs and the absorbance at 390 nm was recorded.

Determination of A_{600}: The bacterial pellet from tube 3 was resuspended in 10 ml sterile distilled water. The absorbance of the solution at 600 nm was recorded.

"a" value: The "a" value was recorded on the external surface of patties by a Minolta Chromameter (CR-200b, Minolta Camera Co. Ltd. Japan). Two different measurements were made on the surface after subjecting the samples to air for a period of 30 min.

Aerobic plate count: The aerobic plate count of the samples was determined by spread plate technique using plate count agar (Difco) (Steinbrugge and Maxcy, 1988). The diluent from the stomacher bag containing meat homogenate was serially diluted in 0.1% peptone solution. From appropriate dilutions, duplicate 0.1 ml aliquot were each spread on plate count agar plates and incubated at 25°C for 48 hr.

Results & Discussion

Regression analysis between bacterial count and each of the measured parameters was performed. FDA hydrolysis (Fig 1), aminopeptidase activity (Fig 3), and A_{600} (Fig 4) increased with increased aerobic plate count of the samples, and r-square values were 0.88, 0.86, and 0.93, respectively. Resazurin reduction time steadily decreased as bacterial count of the samples increased. Regression analysis between these two parameters revealed an r-square value of 0.90 (Figure 2). The "a" value of the patties declined during storage with increased bacterial count; the regression between bacterial count and the "a" values indicated an r-square value of 0.78 (Fig 5). It was also observed that freezing at spoilage did not affect the measured responses, indicating that the parameters can be effectively used to detect spoilage levels of bacteria in frozen beef. Venkitanarayanan et al. (1993) compared FDA hydrolysis, resazurin reduction, and "a" value in irradiated and

non-irradiated beef and reported that meat contains inherent, although limited hydrolytic activity towards FDA; the major hydrolytic activity was produced by bacterial flora in the non-irradiated meat. In the same study, resazurin reduction time in non-irradiated beef steadily declined as the bacterial count increased, while in irradiated beef, the reduction time did not change with storage. In a recently completed companion study, it was found that FDA hydrolysis, RR, AP activity, A_{600}, but not "a" values could be used as rapid indicators of bacterial load in whole beef cuts (results not shown).

Ayres (1960) demonstrated that meat spoils when the bacterial count reaches about 10^7-10^8 CFU cm^{-2}. In the present study, we found that, at a bacterial load of log 7.5/g, the FDA hydrolysis yielded an absorbance of ca. 0.36 at 490 nm, and resazurin reduction occurred in 1.75 hrs. At the same bacterial load, the aminopeptidase test yielded an absorbance of 0.15 at 390 nm, while the absorbance at 600 nm was 0.20. This study indicated that FDA hydrolysis, resazurin reduction, aminopeptidase activity, and absorbance at 600nm may be potentially used as rapid indicators of bacterial load in ground beef. However detailed studies involving a larger sample size and more replications are needed to confirm the reliability of these tests. Although the regression between bacterial count and "a" values revealed a satisfactory r-square value, the reliability of meat color as an index of bacterial load needs further research, as the basis for potential bacterial-mediated discoloration is not well characterized.

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