Journal of Food Protection, Vol. 68, No. 7, 2005, Pages 1414–1420

DNA Comet Assay for the Detection of Time-Temperature Abuse during the Storage of Poultry

CEDRIC FAULLIMEL,[1] SAÏD ENNAHAR,[1] DALAL AOUDE-WERNER,[2] PATRICK GUTERL,[1] AND ERIC MARCHIONI[1]*

[1]*Laboratoire de Chimie Analytique et Sciences de l'Aliment (UMR 7512), Faculté de Pharmacie, Université Louis Pasteur, 74, route du Rhin, F-67400 Illkirch Graffenstaden, France; and* [2]*Aérial, 19, rue de Saint-Junien, F-67300 Schiltigheim, France*

MS 04-367: Received 5 August 2004/Accepted 14 March 2005

ABSTRACT

Effects of abusive storage conditions on the quality of fresh chicken were studied by detecting DNA damage to breast fillets and liver with the neutral comet assay. Chilled samples were kept at 4°C for prolonged periods, whereas frozen samples were exposed to temperatures of 4°C, representing inadvertent thawing, and 20°C, representing extreme abuse in the distribution chain. Comets' mean tail moment distributions reflected the increasing patterns of DNA damage, but the differences of values between close levels of treatment were sometimes insignificant. The design of the DNA damage index, integrating the distribution of mean tail moments over three trials, provided values significantly different, which allowed a more precise discrimination between samples according to the treatment levels. Considering the background level of DNA damage in control cells, a DNA damage index value of 50 μm was set as a limit for the detection of abusive storage. Temperature abuse could be detected after 7 and 22 h of exposure at 4°C for liver and breast, respectively. These durations were by far shorter (1.5 and 2.5 h, respectively) when the temperature was increased to 20°C. As for chilled storage, its damaging effects could be detected after 1.5 and 2.5 days for liver and breast, respectively. Liver cells were more sensitive to abusive conditions than breast muscle cells. The comet assay's detection limit was applicable to samples that were still considered of good quality with regard to the microbiological shelf life, thereby showing its high sensitivity as a rapid test for assessing the quality of fresh chicken.

The quality and shelf life of poultry meat is related to the load of bacteria present on the carcass before slaughter, efficiency of processing, and the conditions and time of storage that allow bacterial proliferation before consumption *(10)*. Control of the storage temperature is vital in maintaining the quality of poultry meat at an adequate level throughout the distribution chain. Temperature affects the growth of aerobic mesophilic bacteria, proteolytic bacteria, hydrogen sulfide–producing bacteria, *Enterobacteriaceae*, and clostridia, the microbial groups most likely to cause spoilage *(22)*. As the temperature is decreased below the optimum for growth, generation times and lag times are extended, and the microbiological shelf life can be considerably improved. However, many of the major food spoilage and food poisoning microorganisms of concern are cold-adapted psychrotrophic bacteria that are able to grow at chill temperatures *(20)*. Therefore, the storage time is also important because extended storage can give an opportunity for some pathogens like *Listeria* (able to grow near 0°C) to gradually multiply and reach high levels.

Microbiologically, poultry products are considered unfit for consumption when aerobic mesophilic counts (AMCs) reach 10^7 CFU/g *(11, 21)*. Although this analysis is useful in assessing the seasonal and flock-to-flock variations of bacteria on the carcasses, it remains cost prohibitive and time consuming. Instead, efforts should be directed toward controlling the effective management of the cold chain because the microbiological quality of refrigerated and frozen chicken can be dramatically improved when temperature is maintained at values representing an ideal, unbroken cold chain *(22)*. To date, only time-temperature indicators attached to the package surface can be used to help reveal a rise in the cold chain temperature or an abusive storage. The application of time-temperature indicators for the sensory and microbiological quality control of chicken cuts under various temperature conditions was recently addressed by Smolander et al. *(22)*, who, despite the positive results, found that one of the difficulties with these indicators was to adjust their endpoints (complete change) to the product's shelf life. Also, time-temperature indicators can only work when activated and while in contact with the product. Therefore, a rise in the cold chain temperature occurring before contact or activation, or after the removal of the time-temperature indicators, would remain undetected.

DNA degradation, on the other hand, which naturally occurs in living tissues after death, can be taken advantage of to detect a rise in the cold chain temperature in the food itself or an abusive storage before bacteria can reach spoilage or hazardous levels. This process is in fact accelerated by increased temperatures, reduced under chilled conditions, and practically stopped at temperatures of less than −26°C *(6, 19)*. DNA integrity can be directly assessed by the single cell gel electrophoresis assay (also called the DNA comet assay), which is a rapid, simple, and low-cost

* Author for correspondence. Tel: (33) 3 90 24 43 26; Fax: (33) 3 90 24 43 25; E-mail: eric.marchioni@pharma.u-strasbg.fr.

screening test. This method, described in 1984 by Östling and Johanson *(18)*, is now widely accepted and is being used to estimate DNA fragmentation in a variety of research areas, such as genotoxicity, radiotherapy, carcinogenesis, environmental biomonitoring, and detection of irradiated food *(3–5, 7, 9, 12, 13, 16, 17)*. In this assay, cells with damaged DNA embedded and lysed in agarose gel migrate, leaving a cometlike electrophoretic trail with a head corresponding to the cell (DNA of high molecular masses) and a tail corresponding to fragments of DNA. The length and shape of the comets reflect the amount of DNA damage in the cell, and intact cells appear nearly circular or with very short tails.

The DNA comet assay is a simple, rapid, and inexpensive technique that can be an effective tool to allow official control agencies to monitor the realization of an unbroken cold chain. In this work, we describe the application of the comet assay in assessing DNA damage in frozen and chilled fresh chicken subjected to temperature abuse and extended storage, respectively.

MATERIALS AND METHODS

Chemicals. The chemicals, of analytical grade when available, used in these experiments were purchased from the following suppliers: low-melting agarose (LMA) from Biorad (Hercules, Calif.); sodium chloride, sodium hydroxide, boric acid, potassium chloride, sodium monohydrogen phosphate, and sodium dodecylsulphate from Merck Chemicals (Darmstadt, Germany); ethylenediamine tetraacetic acid disodium salt dihydrate (EDTA), Tris base, and potassium dihydrogen phosphate from Sigma (Saint-Quentin, France); and Sybr Gold staining solution from Molecular Probes (Leiden, The Netherlands). Ultrapure water was produced by a Milli-Q PLUS filtration system (Millipore, Saint Quentin, France). Glass- and plasticware were cleaned and sterilized (120°C, 20 min) before use.

Samples. Fresh chicken liver and breast fillets, with bone out and skin off, were selected from the production line of a commercial processor. Within the 3 h following slaughter, they were cut into slices (weight 10 g, height 1 cm, width 3 cm, depth 3 cm), wrapped in polyethylene bags, and frozen (−18°C) for 1 week. Some samples were chill-stored in a refrigerator at 4°C for up to 9 days. Before analysis, frozen samples were thawed and held either at 4°C for 2, 6, 12, 24, and 48 h or at 20°C for 0.5, 1, 2, 4, 6, and 8 h. To be completely defrosted (temperature above 0°C inside), a sample of chicken breast had to be held 70 and 24 min at 4°C and 20°C, respectively, whereas a sample of chicken liver required only 30 and 8 min at 4°C and 20°C, respectively. Chilled samples were analyzed after 1, 2, 4, 6, 5, 7, 8, and 9 days of storage. Temperature was monitored with a calibrated Testo 901 thermometer (Testo, Forbach, France) equipped with a 1-mm-thick Pt 100 heat sensor. The sensor was placed on the surface of the product for measurement of the outer temperature (underneath a plastic bag) or stuck into the core of the chicken sample for measurement of the inner temperature. During analysis, samples were kept on an ice bed and handled with gloves.

Cell preparation. Chicken cells were isolated from a 0.3-g surface sample cut out with a scalpel and mixed with 5 ml of phosphate-buffered saline (0.1 M NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.4) for 5 min on a magnetic stirrer (500 rpm). The suspension was filtrated through an absorbent layer of gauze, then through a 250-μm-pore-size disposable nylon sieve cloth, and allowed to settle for 5 min. Cells, taken from the upper phase, were kept on ice until analysis.

Slide preparation. The neutral comet assay described by Koppen and Cerda *(15)* was used with slight changes. Partially frosted microscopic slides (Marienfeld Glassware, Lauda Koenigshofen, Germany), prewashed overnight in methanol, were each covered with 50 μl of 0.5% LMA at about 60°C in water (wt/wt). They were immediately covered with a coverslip (24 by 60 mm, Knittel Glaser, Braunschweig, Germany) and kept at room temperature for about 5 min to allow the agarose to solidify. After gently removing the coverslip, 100 μl of the LMA cell suspension (100 μl of the cell suspension diluted in 600 μl of warm [60°C] 0.75% LMA in phosphate-buffered saline [wt/wt]) was rapidly pipetted onto the first agarose layer (used to promote the adherence of this second layer) and spread evenly with a second coverslip while avoiding air bubbles. The slides, three per sample, were maintained on an ice-cold flat tray for 5 min to solidify before the coverslip was removed. The slides were immersed in 150 ml of a lysing solution containing 2.5% (wt/vol) sodium dodecyl sulfate in TBE buffer 45 mM (45 mM Tris base, 45 mM boric acid, and 1 mM EDTA, pH 8) for 10 min at room temperature, then washed for 5 min in TBE buffer 45 mM. This step and all subsequent steps were conducted under dimmed light, with the slides often covered with aluminum foil to prevent the occurrence of additional DNA damage.

Electrophoresis. The slides were drained and placed in a horizontal gel electrophoresis tank side by side, avoiding spaces and with the agarose ends facing each other, nearest the anode. The tank was filled with fresh electrophoresis solution (TBE buffer 45 mM) to a level approximately 2 to 5 mm above the slides. Electrophoresis was conducted on an ice-cold flat tray for 2.5 min at 2 V/cm with a GD 61D compact power supply (Sebia, Paris, France). After electrophoresis, the slides were taken out of the tank, washed in 150 ml of water for 5 min, and dried in open air for 1 h.

Staining and slide scoring. The slides were immersed in 150 ml of Sybr Gold staining solution (diluted 30,000 times in phosphate-buffered saline, pH 7.4) for 10 min at 4°C and finally washed for 5 min at 4°C in 150 ml of water. They were placed in a humidified airtight container to prevent drying of the gel and analyzed within 3 to 4 h. For visualization of DNA damage, slides were examined at ×400 magnification with a ×40 objective on a fluorescence microscope (Nikon, Tokyo, Japan) equipped with a B-2A filter (ex: 420 to 490 nm, em: 510 nm) and a monochrome camera. Test runs were replicated three times, with three samples analyzed per measurement. For each sample, three slides were prepared, images of 100 randomly selected cells (i.e., ~33 cells from each of triplicate slides) were analyzed from each sample, and the DNA damage was assessed.

Image analysis. An image analyzer capable of measuring comet length, DNA content in head and tail coupled to the Comet Assay III software (Perceptive Instrument, Suffolk, UK) was used. Comet's tail moment (tail length multiplied by the ratio of DNA amounts in the tail and in the whole comet) was determined for 100 cells as described by Anderson et al. *(1)*. Mean frequency distributions of tail moments were determined and represented in histograms. An additional parameter we called the DNA damage index (DDI), which integrates the distribution of mean tail moments over three trials, was designed. It is defined as the average tail moment (μ) plus three times the standard deviation (σ) and is meant to give an estimation of the tail moment value corresponding to a certain level of DNA damage ($DDI = \mu + 3\sigma$).

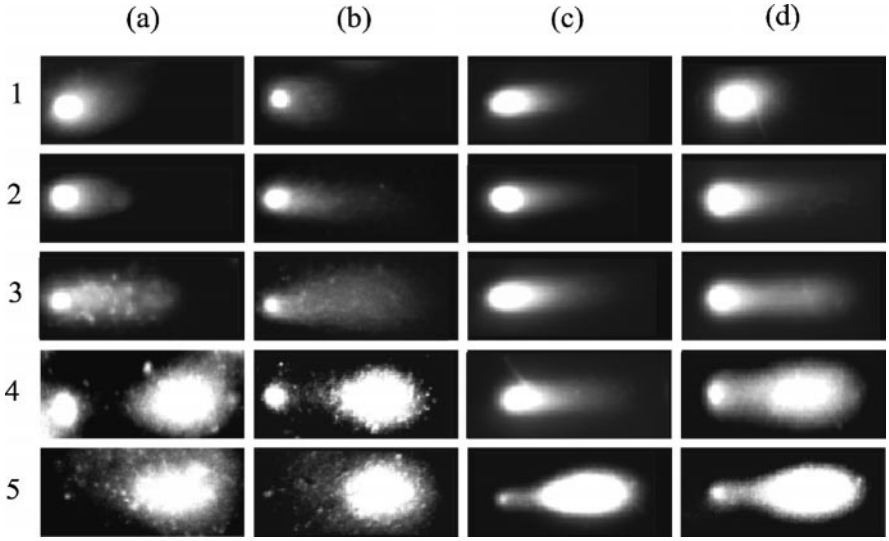

FIGURE 1. *Typical DNA comets with cells from control frozen chicken samples (1) and thawed samples: liver at 4°C (a) for 2 h (2), 6 h (3), 12 h (4), and 24 h (5) and at 20°C (b) for 1 h (2), 2 h (3), 4 h (4), and 6 h (5); breast at 4°C (c) for 6 h (2), 12 h (3), 24 h (4), and 48 h (5) and at 20°C (d) for 2 h (2), 4 h (3), 6 h (4), and 8 h (5). Electrophoresis: 2 V/cm for 2.5 min, migration from left to right. DNA staining done with Sybr Gold solution. Observation at ×400 magnification.*

Microbiological analysis. Enumeration of AMCs was performed by the aerobic plate count technique according to the ISO 4833 method *(2)* as follows: chicken samples (25 g) were placed in separate sterile laboratory blender jars with 225 ml of buffered peptone water (Bio-Rad, Marne-la-Coquette, France). Diluent and sample were blended for 2 min and serially diluted if necessary, and aliquots (1 and 0.1 ml) of appropriate dilutions were pour plated, in duplicate, on plate count agar (Bio-Rad). Plates were incubated at 30°C for 72 h. Numbers (CFU per gram) from chicken samples were determined. Both breast muscle and liver were analyzed as three replicates per analysis.

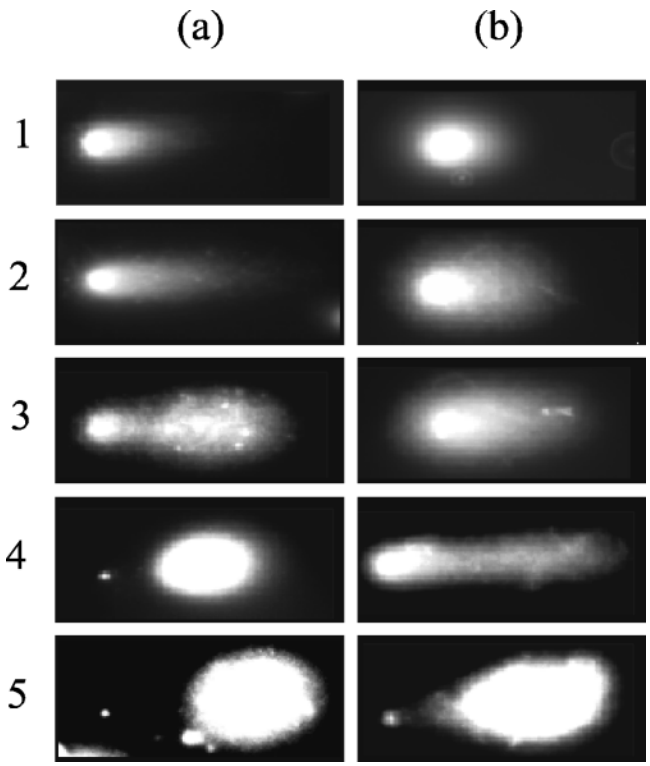

FIGURE 2. *Typical DNA comets with cells from control chicken samples (1) and samples chill-stored at 4°C: (a) liver for 2 days (2), 4 days (3), 7 days (4), and 8 days (5); (b) breast for 2 days (2), 4 days (3), 7 days (4), and 9 days (5). Electrophoresis, staining, photography, and magnification as in Figure 1.*

RESULTS

Effects of a rise in temperature on DNA damage. Figure 1 shows representative undamaged and migrated liver and breast cells from samples thawed and held at 4 and 20°C for various periods of time. Cells from liver samples held for 2 h at 4°C and 1 h at 20°C (Fig. 1a [3] and 1b [2]) showed short-tailed comets with relatively little DNA degradation. For samples that underwent exposure for up to 6 h at 4°C and 2 h at 20°C, comets continued stretching, with tails of increasing lengths but of even width (Fig. 1a [3] and 1b [3]). For longer exposure times, tail length stopped increasing, although DNA fragments continued migrating from head to tail (Fig. 1a [4] and 1b [4]). As a consequence, the tail started widening and increasing in size while gradually separating from the head. After 24 h at 4°C and 6 h at 20°C (Fig. 1a [5] and 1b [5]), almost no DNA was left in the head, which was reduced to the size of a small dot (the nucleus); the tail appeared as a cloud, far from the head. The density of fragmented DNA in the tail of the comets gave an indication of the extent of exposure to high temperatures because it increased with time after thawing. Similar migration patterns of DNA with distinct comets were observed with chicken breast cells (Fig. 1c [2 through 5] and 1d [2 through 5]). The trends of DNA degradation were not different from liver cells, although the comets were somewhat thinner and longer in shape. It also appeared that the overall damaging effect of exposure to high temperatures after thawing was much quicker and more pronounced with liver cells.

Effects of chill storage on DNA damage. For the assessment of the effects of chill storage on tissues of chicken breast and liver, DNA damage levels in samples kept at 4°C were compared with fresh samples, as evaluated by the comet assay. DNA degradation increased with storage time, which was reflected in the shape of the comets. For liver cells, lengths of comets produced by chill storage increased during the first 4 days of storage (Fig. 2a [1 through 3]). This phase lasted 7 days with breast cells, although the patterns of DNA damage in the two kinds of tissues were

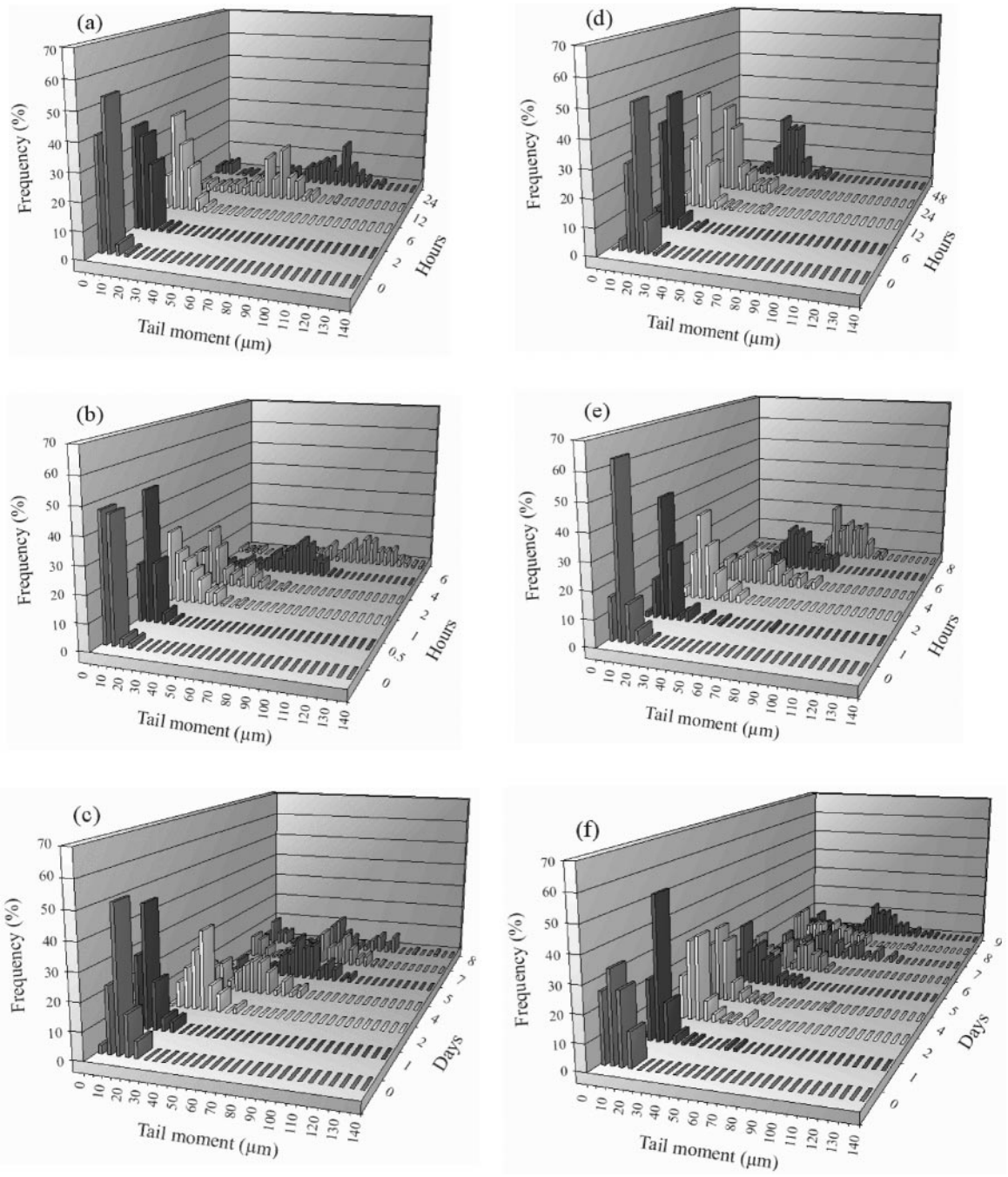

FIGURE 3. *Change in frequency distribution of tail moments of 100 migrated cells extracted from chicken samples: frozen liver thawed at 4°C (a) and 20°C (b) and chill-stored liver at 4°C (c); frozen breast thawed at 4°C (d) and 20°C (e) and chill-stored breast at 4°C (f).*

not different (Fig. 2b [1 through 4]). For longer storage times, comets' lengths remained almost unchanged, while their tails markedly swelled and gradually separated from their heads. By days 8 and 9 for liver and breast cells, respectively, almost all the DNA was damaged, and fragments migrated to the tail.

Distribution patterns of tail moment frequencies. Comets' tail moments were determined for 100 cells. The mean frequency distributions of comets (% comets) according to the ranges of tail moments were analyzed. Trends for breast fillets and liver samples thawed and held at 4 and 20°C and samples chill-stored at 4°C are given in Figure 3. Compared with samples that had undergone thawing or chill storage, which generally contained high numbers of cells with DNA damage, controls contained almost exclusively intact cells and displayed a narrow peak centered around low tail moment values (10 to 20 μm). For frozen liver samples, it appeared that the amount of DNA damage correlated well with time after thawing: the longer the duration, the stronger the damaging effect and the higher the frequency of DNA comets with high tail moments (Fig. 3a and 3b). Similarly, there was a clear association between temperature and the amount of DNA damage because, for the same exposure periods, the relative numbers of comets

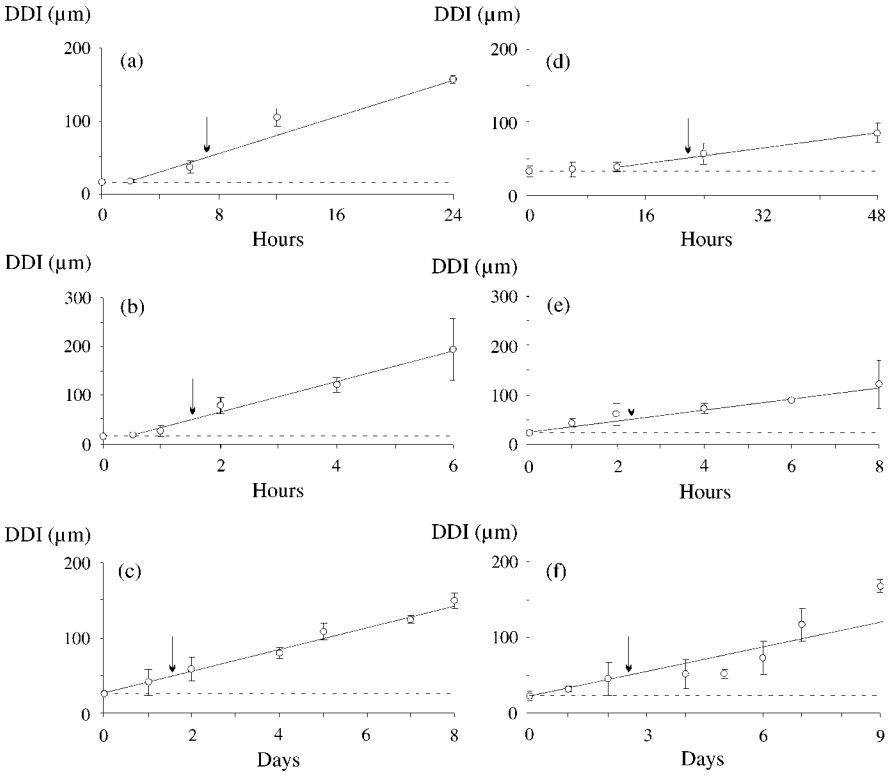

FIGURE 4. *Change in DNA damage index values for cells extracted from chicken samples: frozen liver thawed at 4°C (a) and 20°C (b) and chill-stored liver at 4°C (c); frozen breast thawed at 4°C (d) and 20°C (e) and chill-stored breast at 4°C (f). The dashed line corresponds to the background DNA damage level. The arrow indicates the time necessary to achieve a DDI value of 50 μm.*

with higher tail moments were by far greater at 20 than at 4°C. In parallel to the increased effects of time-temperature after thawing, the patterns of frequency distribution of comets became gradually scattered over wider ranges of tail moments. However, thawed liver samples that were held for 12 h at 4°C and 4 h at 20°C could be discriminated from the unthawed samples because of the relatively larger rates of cells with high tail moments (centered around 65 μm). The patterns of tail moment frequencies resulting from thawing of chicken breast were similar to those observed with liver tissues. There was a clear association between the time and temperature after thawing and the extent of DNA damage (i.e., tail moments [Fig. 3d and 3e]). As expected, the increase in the frequencies of cells with comets was slower in breast tissues than in liver tissues, especially at 4°C, at which samples had to remain for 48 h before they could be differentiated form the unthawed samples. After thawing at 20°C, however, 6 h was sufficient for a clear discrimination. With regard to chill storage, the fresh samples contained very distinct and pronounced round intact cells of nearly the same sizes. Liver samples kept for 4 days and breast samples kept for 5 days at 4°C could be distinguished from the fresh samples because of relatively higher rates of cells with large tail moments. However, as for thawing treatments, extended storage led to widely scattered comet patterns, with the presence of apparently undamaged cells even in the case of samples chill-stored up to 8 or 9 days.

Detection limits for the DNA Comet Assay. As shown in Figure 4, comets with higher DDIs (the distribution of tail moments corresponding to more damaged DNA) increased with exposure times after thawing and with storage times. The increasing patterns of DNA damage with time were much more regular compared with the results of the tail moments distribution. Increases were sharper with liver samples than with breast samples, which reflects the higher damaging effect of exposure to thawing temperatures and extended chill storage on liver tissues. A background DDI level was established as the DNA damage corresponding to control samples (Fig. 4). When the DDI was above this level, the sample was considered to be differentiable from the control. In this study, the detection limit for the comet assay (DDI above the background level) was set at a value of ca. 50 μm. In the case of thawing, the detection limit was reached at 7 and 22 h at 4°C and at 1.5 and 2.5 h at 20°C for chicken liver and breast samples, respectively. As for chill storage, the detection limit coincided with 1.5 days for liver and 2.5 days for breast.

Microbiological quality. Aerobic mesophilic bacterial counts after thawing and during storage test runs are shown as a function of time in Figure 5. The results are averages of three replicate samples. Differences in initial microbial loads between the two types of chicken tissues were highlighted, with liver samples containing 10^4 to 10^5 CFU/g and breast samples containing approximately 10^3 CFU/g. With respect to growth rates, there were no major variations according to temperature as far as thawed breast muscle is concerned because the total flora contents remained almost unchanged over time at 4 and 20°C. For liver however, samples thawed at 20°C showed a moderate increase of the bacterial counts, reaching up to ca. 10^6 CFU/g after 4 h from an initial load of ca. 10^4 CFU/g, whereas counts remained unchanged at 4°C. During chill storage at 4°C, aerobic mesophilic populations increased steadily until microbial counts of 8 log were reached with liver and breast after 8 and 9 days, respectively (Fig. 5c).

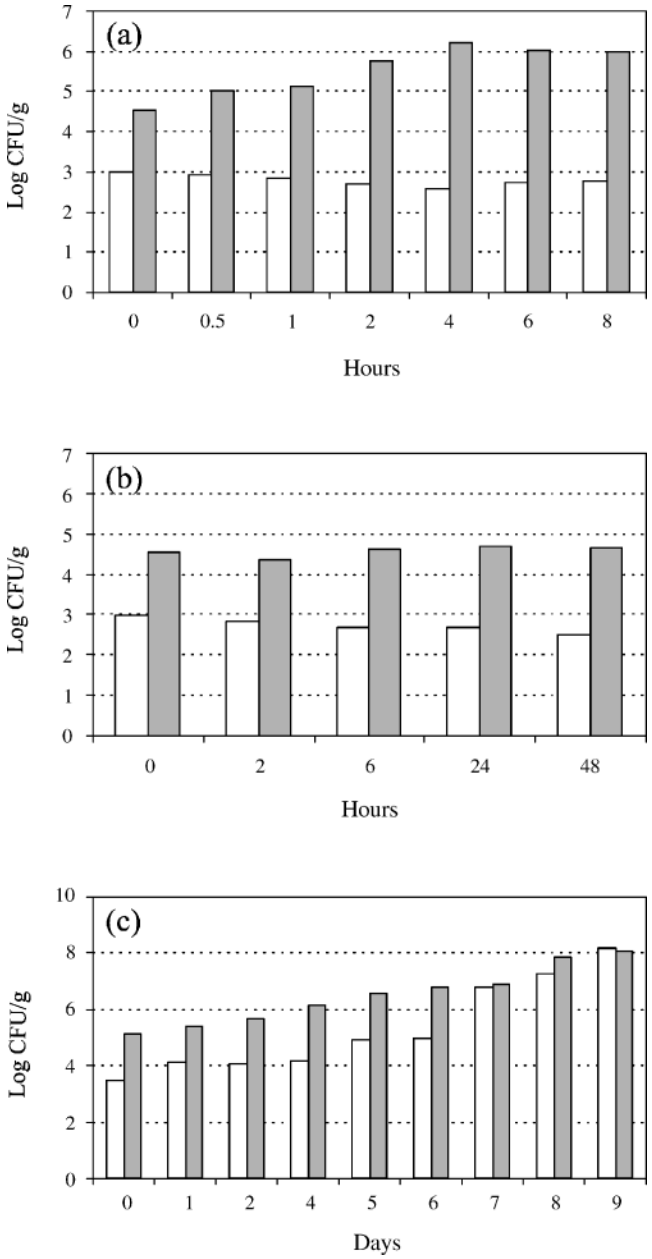

FIGURE 5. *Populations of aerobic microorganisms in chicken breast fillet (□) and liver (▨) after thawing at 20°C (a) and 4°C (b) and during storage at 4°C (c); mean and range. Analysis was done in three replicate measurements at indicated treatment times.*

DISCUSSION

Observation of thawed and chill-stored chicken liver and breast cells showed DNA migration patterns in the comet shapes. These patterns, which reflected the level of DNA damage, correlated well with the extent of postthawing exposure and storage. Control samples could usually be discerned from thawed and chill-stored samples at a glance on the basis of comet lengths. However, the presence in the DNA migration process of a second phase, in which the comet's length remains unchanged, showed that the length of the comet alone is not a sufficient parameter to describe the extent of DNA degradation. The use of the tail moment, which takes into account the distribution of DNA over the whole comet, allowed the monitoring of phase 2 and therefore the overall DNA damage. This helped improve the sensitivity of the comet assay. The frequency of higher tail moments increased with time for both thawing and chill storage test runs. Also, the increase of thawing temperature from 4 to 20°C resulted in higher tail moment frequencies (Fig. 3).

Nonetheless, because of the generally wide distribution of tail moment frequencies for advanced treatment levels, patterns often overlapped with each other, and precise discrimination was difficult unless between largely different levels of treatment. For this reason, the DDI, which integrates mean tail moment frequencies over three trials, was thought to be more indicative of a certain level of DNA damage. Mean DDI values increased gradually with postthawing and storage times (Fig. 4), a trend that was not as clearly shown through the results of tail moment frequency distributions. This further supported the DDI being used as a criterion to assess DNA damage levels.

The detection limit, corresponding to DDI $\approx$ 50 μm, was reached far earlier with chicken liver tissues than with breast tissues. Also, compared with a determination from tail moment frequency patterns, DDI calculations considerably improved the efficiency of detection (e.g., with breast tissues: earlier detections by up to 3.5 h at 20°C and 26 h at 4°C for postthawing exposure, and 2.5 days for chill storage). The overall detrimental effects of exposure to thawing temperatures and chill storage were more considerable on liver tissues than on breast tissues, although the patterns of DNA damage were not different (Figs. 1 through 5). The explanation most certainly lies in differences in the composition of the tissues, the liver being the site of higher metabolic activity because of its ability to express a plethora of microsomal enzymes, both quantitatively and qualitatively *(8, 14, 19).*

The microbiological shelf life of chicken is determined on the basis of the AMC: the product has good quality if the AMC is below 10^6 CFU/g, satisfactory quality if the AMC is between 10^6 and 10^7 CFU/g, and unacceptable quality if the AMC is above 10^7 CFU/g *(21).* In this study, analysis highlighted a difference in initial bacterial loads between breast fillets and liver, with AMCs up to 2 log higher in liver, the latter certainly being more prone to contamination following slaughter. With thawing test runs, apart from a moderate increase in liver at 20°C, the AMC of temperature-abused chicken samples was not remarkably affected, indicating that a time interval of 48 h at 4°C did not result in a product unfit for consumption. During chill storage however, the quality was found to be deteriorating in liver, although still satisfactory, starting on day 4, whereas in breast muscle, this coincided with day 7. This means, in practice, a difference of 3 days in the shelf life between the two kinds of tissues, which could be ascribed to the unequal original microbial loads. By day 8, AMCs in both sets of samples had exceeded 10^7 CFU/g, indicating that they could be considered spoiled *(21).*

Results of this study showed a consistency between trends that occur in the microflora and the DDI values on stored fresh poultry in the sense that a microbial spoilage of chicken is necessarily reflected by higher DDIs. Never-

theless, one should not see a direct link between the presence of bacteria and DNA damage. In fact, when the contamination of a product is the result of bacterial growth following improper storage conditions, this would be confirmed by DNA damage, as opposed to a freshly contaminated product, which might still have intact DNA. On the other hand, although measurement of DDIs after thawing indicated diminishing meat quality, the time did not allow for bacterial spoilage of the meat, particularly at 4°C. Therefore, it appears that an increase in temperature over the thawing point lasting up to 48 h might not be detected by microbial analysis. This highlights the potential of the comet assay as a tool for the assessment of quality and the detection of small time-temperature abuses of the cold chain, before the bacterial numbers can reach spoilage levels.

In this study, we provide evidence of DNA damage in thawed and chill-stored chicken liver and breast compared with controls. The analysis of DNA comet tail moment distribution with DDI calculations allowed the discrimination of the extent of exposure to thawing temperatures and chill storage. It is assumed that the DDI would integrate the cumulative time-temperature history of the product starting with processing and throughout the distribution chain. The DNA comet assay therefore offers considerable promise for monitoring the quality and detecting rises in cold chain temperature and abusive storage of a wide variety of refrigerated foods. The development of such simple and rapid tests contribute toward the simplification of food control, thereby enhancing consumer confidence in the proper surveillance of food quality.

REFERENCES

1. Anderson, D., T.-W. Yu, B. J. Phillips, and P. Schmezer. 1994. The effect of various antioxidant and other modifying agents on oxygen-radical generated DNA damage in human lymphocytes in the comet assay. *Mutat. Res.* 307:261–271.
2. Anonymous. 1991. General guidance for the enumeration of microorganisms—colony-count technique at 30°C. ISO 4833 Microbiology. International Organization for Standardization, Geneva.
3. Anonymous. 2001. Foodstuffs—DNA comet assay for the detection of irradiated foodstuffs—Screening method. European Standard EN 13784. European Committee for Standardization, Brussels, Belgium.
4. Betti, C., R. Barale, and B. Pool-Zobel. 1993. Comparative studies on cytotoxic and genotoxic effects of two organic mercury compounds in lymphocytes and gastric mucosa cells of Sprague-Dawley rats. *Environ. Mol. Mutagen.* 22:172–180.
5. Cerda, H., H. Delincée, H. Haine, and H. Rupp. 1997. The DNA 'comet assay' as a rapid screening technique to control irradiated food. *Mutat. Res.* 357:167–181.
6. Cerda, H., and G. Koppen. 1998. DNA degradation in chilled fresh chicken studied with the neutral comet assay. *Eur. Food Res. Technol.* 207:22–25.
7. Collins, A. R., S. J. Duthie, and V. L. Dobson. 1993. Direct enzyme detection of endogenous oxidative base damage in human lymphocytic DNA. *Carcinogenesis* 14:733–735.
8. Cooper, G. M. 1997. Lysosomes, p. 379–381. *In* G. M. Cooper (ed.), The cell. ASM Press, Washington, D.C.
9. Fairbairn, D. W., P. L. Olive, and K. L. O'Neill. 1995. The comet assay: a comprehensive review. *Mutat. Res.* 339:37–59.
10. Freeman, L. R., G. J. Silverman, P. Angelini, C. Merritt, Jr., and W. B. Esselen. 1976. Volatiles produced by microorganisms isolated from refrigerated chicken at spoilage. *Appl. Environ. Microbiol.* 32: 222–231.
11. Gram, L., L. Ravn, M. Rasch, J. Bartholin Bruhn, A. B. Christensen, and M. Givskov. 2002. Food spoilage—interactions between food spoilage bacteria. *Int. J. Food Microbiol.* 78:79–97.
12. Hartman, A., and G. Speit. 1995. Genotoxic effect of chemicals in the single cell gel (SCG) test with human blood cells in relation to the induction of sister chromatid exchanges (SCE). *Mutat. Res.* 346: 49–56.
13. Khan, A. A., H. M. Khan, and H. Delincée. 2002. Detection of radiation treatment of beans using DNA comet assay. *Radiat. Phys. Chem.* 63:407–410.
14. Koohmaraie, G. M. 1994. Muscle proteinase and meat aging. *Meat Sci.* 36:93–104.
15. Koppen, G., and H. Cerda. 1997. Identification of low-dose irradiated seeds using the neutral comet assay. *Lebensm.-Wiss. Technol.* 30:452–457.
16. Lee, R. F., and S. Steinert. 2003. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. *Mutat. Res.* 544:43–64.
17. McKelvey-Martin, V. J., M. H. L. Green, P. Schmezer, B. L. Pool-Zobel, M. P. De Méo, and A. Collins. 1993. The single cell gel electrophoresis assay (comet assay): a European review. *Mutat. Res.* 288:47–63.
18. Östling, O., and K. J. Johanson. 1984. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. *Biochem. Biophys. Res. Commun.* 123:291–298.
19. Park, J. H., C. K. Hyun, S. K. Jeong, M. A. Yi, S. T. Ji, and H. K. Shin. 2000. Use of the single gel electrophoresis assay (comet assay) as a technique for monitoring low-temperature treated and irradiated muscle tissues. *Int. J. Food Sci. Technol.* 35:555–561.
20. Russell, N. J. 2002. Bacterial membranes: the effect of chill storage and food processing. An overview. *Int. J. Food Microbiol.* 79:27–34.
21. Senter, S. D., J. W. Arnold, and V. Chew. 2000. APC values and volatile compounds formed in commercially processed, raw chicken parts during storage at 4 and 13°C and under simulated temperature abuse conditions. *J. Sci. Food Agric.* 80:1559–1564.
22. Smolander, M., H.-L. Alakomi, T. Ritvanen, J. Vainionpää, and R. Ahvenainen. 2004. Monitoring of the quality of modified atmosphere packaged broiler chicken cuts stored in different temperature conditions. A. Time-temperature indicators as quality-indicating tools. *Food Control* 15:217–229.