Review

Analytical methods for authentication of fresh vs. thawed meat – A review

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Abstract

Proper labeling of meat products is important to ensure fair-trading and to enable consumers to make informed choices. Different investigations indicate that wrong labeling where thawed meat is labeled as fresh meat is present in 8–15% of analyzed samples. Enforcement of regulations requires adequate analytical methods where enzymatic-, DNA based-, spectroscopic-, bio imaging- and sensory techniques constitute the majority of published papers. The molecular changes that these techniques detect are described. The capability of both discrimination between fresh and thawed meat, and determination of frozen storage time are discussed for each of the analytical techniques. The products included in this review are primarily whole meat from Bos taurus (cow), Sus scrofa (pig) and Gallus gallus (chicken). The best analytical choice in the discrimination of fresh vs. thawed meat is concluded to be a combination of analytical methods.

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1. Introduction

Meat products represent a large component of human food and its quality is of concern to consumers, governmental control authorities and retailers. Nevertheless, meat can be an attractive target for adulteration in many ways and one very obvious kind is to illegally sell thawed meat as fresh. The attractiveness is obvious since fresh meat is more valuable than frozen meat.

To enforce regulations and get a comprehensive knowledge of the amount of fraud present, adequate analytical methods are required. A few published investigations have shown that fraud or mislabeling is a problem in the case of thawed meat sold as fresh meat. Laboratories of Appenzell AR and AI, Glarus, and Schaffhausen found that 15% of 43 meat samples collected in Switzerland were wrongly declared as fresh (Gremau, Karlen, & Hulliger, 2002) and a surveillance exercise by the Working Party on Food Authenticity in the United Kingdom found that 8% of 534 samples were mislabeled (Ministry of Agriculture Fisheries & Food, 1996). Furthermore, an Internet search revealed that during recent years numerous cases have occurred where meat has been sold and partly consumed after frozen storage far exceeding the expiry date.

The structural and molecular changes that take place during frozen storage and freeze-thaw cycles are numerous and most frequently are the formation of ice crystals (Smith, 1950) and the resulting local increase in the salt concentration (Lovelock, 1953). The ice crystals are formed from ice nuclei, in a process called nucleation. Nucleation is dependent on the degree of super cooling, viscosity, physical disturbances, interfacial energy and freezing rate (Fennema, 1973). A fast freezing rate results in many extracellular ice nuclei whereas slow freezing produces fewer, extracellular ice nuclei (Martino, Otero, Sanz, & Zaritzky, 1998). The growth of ice crystals from nuclei depends on freezing rate, final freezing temperature, storage time, and pressure throughout the freezing process. Ice crystal growth seems to have an upper size limit, which is reached after a certain time at subzero storage (Martino & Zaritzky, 1988). One consequence of the formation of ice crystals is physical damage to the microstructures, which continues throughout frozen storage and thawing due to a continuous recrystallization.

Ice crystals form as a pure substance, and solutes are therefore concentrated in the liquid phase between the crystals. About 8–10% of the water in animal tissue is unavailable for ice formation (Meryman, 1966). This water allows both chemical reactions and physical movement, and the increase in salt concentration denatures proteins and damages intracellular organelles, probably due to osmotically induced swelling (Farrant, Walter, & Armstrong, 1987). The damage to cell organelles can result in a release of its content, the lysosomes are especially important since they contain digestive enzymes such as lipases, carbohydrates, proteases, nucleases and phosphatases.

The most widely used enzymatic method to distinguish fresh from thawed meat is the β-hydroxyacyl-CoA dehydratase method (HADH) (Gottesmann & Hamm, 1983) that takes advantage of the disruption of mitochondria induced by freezing of whole meat. Disruption of mitochondria also occurs during grinding, which makes discrimination by the HADH and other enzymatic methods impossible in ground meat products. Ground meat is therefore not included in the Section 2.

This review will focus on detection of the major molecular changes that occur during traditional freezing (approximately −18 °C) at atmospheric pressure, of especially beef, pork and chicken whole meat. Other species are included where the literature on beef, pork and chicken is limited.

Different analytical possibilities for the determination of fresh vs. thawed meat authentication and of the actual frozen storage time are described. The analytical methods most often referred to in the literature are discussed and include: enzymatic-, DNA based-, spectroscopic-, bio imaging- and sensory methods. Since no single method is adequate in the authentication of fresh vs. thawed meat, the focus is on the advantages and disadvantages of different methods.

2. Enzymatic methods

The amount of enzymes is increased in meat press juice after freeze-thawing due to damage of cell compartments, and the focus has primarily been on the release of these enzymes from mitochondria and lysosomes. The press juice can be collected either by the application of mechanical force such as a stomacher, or less commonly by centrifugation.

To discriminate between fresh and thawed meat numerous enzymatic methods take advantage of this enzymatic release. Particularly the HADH method applied to press juice has been widely used and discussed (Billington, Bowie, Scotter, Walker, & Wood, 1992; Chen, Yang, & Guo, 1988; Gottesmann & Hamm, 1983; Ministry of Agriculture Fisheries & Food, 1996; Toldra, Torrero, & Flores, 1991). Originally, one spectrophotometric and one color
test method based on the same reaction (1) were published (Gottesmann & Hamm, 1983).

\[
\text{Acetoacetly-CoA + NADH + H}^+ \rightarrow \beta - \text{hydroxybutyryl-CoA + NAD}^+ \tag{1}
\]

The spectrophotometric method measures the conversion rate of NADH to NAD\(^+\) by monitoring the decrease in absorption at 340 nm. Gottesmann and Hamm (1983) reported threshold HAD activity values for individual species, and values above these threshold values are indicative of a freeze-thawed treatment.

The color test method is based on a subsequent chemical reaction (2) that relies on the color difference between the oxidized and the reduced form of melodolabue, which are blue and colorless, respectively (Gottesmann & Hamm, 1983).

\[
\text{NADH + H}^+ + \text{melodolabue(oxidized)} \rightarrow \rightarrow \text{NAD}^+ + \text{melodolabue(reduced)} \tag{2}
\]

The color test method has also been used with substitution of melodolabue by resazurin resulting in increased color stability (Chen et al., 1988). Both the spectrophotometric and the color test methods are applicable to beef, veal, pork, mutton/ham, game and poultry but require that the meat gas been frozen to \(-12^\circ\text{C}\) or below (Gottesmann & Hamm, 1983) and has not been ground.

The spectrophotometric method (Gottesmann & Hamm, 1983) with minor modifications was applied to chicken breast press juice in a collaborative trial (Billington et al., 1992) involving eight laboratories. The collaborative trial showed that discrimination between fresh and thawed chicken breast meat was possible at \(-18^\circ\text{C}\) but not at \(12^\circ\text{C}\). However the reproducibility was poor and the importance of anhydrous storage of NADH could be a critical factor and a possible explanation for the low reproducibility.

Sen and Sharma (2005) studied the activity of acid phosphatase (ACP), alkaline phosphatase (ALP), lipoamide dehydrogenase (LPDH), lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) in the press juice from whole goat meat. The goat samples were stored at \(-18^\circ\text{C}\) for 0, 7, 15, 30, 60 and 90 days. Two different thaw treatments were performed and the press juice was collected from sub samples after centrifugation. The ACP enzyme activity showed a gradual increase during frozen storage with the exception of day 15. The LPDH activity increased during the first 30 days but decreased over the following 60 days. An initial increase in the activities of ALP, LDH and SDH was observed but no correlation with storage time was seen. Except for GOT and GPT which only showed a marginal increase in activity, the above enzymes are possible candidates for fresh vs. thawed meat discrimination. However, proteases are released from lysosomes during freezing and it is possible that proteolytic degradation of individual enzymes could cause a decrease in their activities in the press juice. This would complicate the discrimination between fresh and long term frozen meat when the juice is analyzed.

Different workers (Ellerbroek, Lichtenberg, & Weise, 1995; Toldra et al., 1991) have used the API-ZYM system, which consists of semi-quantitative analysis for the simultaneous measurement of 19 different enzymes. Toldra et al. (1991) investigated the press juice from pork and showed that the enzymatic activity of esterase–lipase, \(\beta\)-glucuronidase and \(\alpha\)-glucosidase differed significantly between fresh and frozen (10–60 °C) thawed pork, but no significant differences were observed at temperatures ranging from 10 to 60 °C or with storage time (11–54 days at \(-18^\circ\text{C}\)). Another study on fresh and thawed meat using the API-ZYM system found significant differences in the activities of N-acetyl-\(\beta\)-glucosaminidase and \(\beta\)-galactosidase in the press juice from fresh and thawed pork (Ellerbroek et al., 1995). Only N-acetyl-\(\beta\)-glucosaminidase differed significantly in beef. They concluded that the method is not adequate for the determination of unknown pork and beef samples since no fresh reference sample from the same animal is normally available. Furthermore, they experienced both unclear and false positive results.

Methods that rely on the release of constituents from cell organelles are all dependent on a freezing procedure that actually damages the cell organelles. Most studied is the HAD method that unfortunately requires a temperature of \(-12^\circ\text{C}\) or below to release the HAD. One can therefore not conclude that a low HAD result (<6 U/ml for pork) means that the meat has not been frozen, and if fresh vs. thawed meat discrimination is the issue, other methods capable of operating at temperatures just below 0 °C must be used.

3. DNA based techniques

The DNA molecule is degraded postmortem by endonucleases, exonucleases, hydrolysis, oxidation and alklylation. DNA base composition, DNA topology, ionic environment and the presence of histone like proteins influence genomic stability in microorganisms (Grayling, Sandman, & Reeve, 1996) and probably also play a role in the degree of postmortem DNA degradation in animal cells.

The chemical degradation is dependent on temperature and is basically described by the Arrhenius equation

\[ k = A e^{-\frac{E_a}{RT}} \]

The equation shows the dependence of the rate constant \(k\) of chemical reactions on the temperature \(T\) and activation energy \(E_a\). \(A\) is the pre-exponential factor and \(R\) is the gas constant.

It is frequently assumed, especially in reports on fossil DNA, that DNA strand breaks produced by the \(\beta\)-elimination process that follows depurination, account for one of the most important postmortem DNA damages (Lindahl,
A recent contradictory study showed that interstrand cross links accumulate approximately 100 times faster than single strand breaks under frozen conditions (between -9 and -12 °C) (Hansen et al., 2006). Since numerous degradative mechanisms are involved, and the single most important one is obviously not unambiguously established, a robust analytical method should detect several kinds of DNA damage.

The diphenylamine method (Schneider, 1957) was used to measure the DNA content in ground bovine thymus every second month during 20 months at -12 and 30 °C, and no DNA decrease was observed (Hajduk, 1999). This indicates that no major microbial digestion takes place, which is to be expected at subzero temperatures.

A neutral sucrose gradient centrifugation assay in a study on *E. coli* showed that dsDNA breaks increased after one freeze-thaw cycle and through 12 months storage at -16 °C (Grezz, Hammer, Robnett, & Long, 1980).

More interesting is the Comet assay (single cell gel electrophoresis) (Ostling & Johanson, 1984; Singh, McCoy, Tice, & Schneider, 1988), which is a simple method based on electrophoresis of lysed cells embedded in agarose on a microscopic slide. The shape of the electrophoresed DNA resembles comets and is studied by fluorescence microscopy. The intensity of the comet tail relative to the comet head reflects DNA damage (Collins, 2004). The detection of DNA damage is dependent on the pH used in the assay. The alkaline Comet assay denatures the DNA to a greater extend than the neutral Comet assay and therefore enables the assay to detect more diverse kinds of DNA damage (Collins, 2004).

A correlation between DNA damage and different refrigerated and freezing conditions of vacuum packaged whole beef was found by use of the neutral Comet assay and a relative damage index was developed (Park et al., 2000). Another study confirmed the usefulness of the Comet assay showing DNA damage increased during six freeze-thaw cycles, and during 60 days storage at -20 °C, except for a slight decrease at day 30 (Park et al., 2000). The results showed discrimination when the comparison was made between fresh and thawed meat from a common origin (Park et al., 2000). Cerda and Kopp (1998) also used a neutral Comet assay and found that fresh whole chickens from different producers or retailers showed similar comet patterns. The above studies (Cerda & Kopp, 1998; Park et al., 2000) suggest it might be possible to discriminate between fresh and thawed samples without reference samples from the same origin.

More recent technologies such as real-time PCR could perhaps be applied to fresh vs. thawed discrimination. It is probably not the most suitable technique for measuring fragmentation of DNA, as only short sequences are amplified. However, PCR are influenced by fragmentation of DNA and interstrand croslinks, which causes the *Tag* polymerase to arrest (O’Brien, Xu, & Patierno, 2001), and a correlation between threshold cycle values and freeze-thaw cycles has been shown in a real time PCR study where DNA in solution was examined (Bellete, Flati, Hafid, Raberin, & Tran Manh, 2003).

### 4. Spectroscopy

Temperature fluctuations and freeze storage induce different physical alterations that result in color changes due to changes in the hemoglobin environment (Deman, 1999). Also the capacity to bind water and the water distribution is altered during freezing. These changes can be detected by various spectroscopic techniques such as visible and near infrared (NIR) spectroscopy, and nuclear magnetic resonance (NMR).

#### 4.1. Ultraviolet and near infrared spectroscopy

Two-dimensional visible/near infrared reflectance spectroscopy was used to correlate frozen storage temperatures of 525 whole chicken breasts with spectral intensities (Liu, Barton, Lyon, Windham, & Lyon, 2004). Chicken samples were stored for 7 days at 4, 0, -3, -12, and -18 °C followed by 7 days at -18 °C and then analyzed at 4 °C. The two-dimensional visible correlation spectra showed single peaks around 445, 475, and 560 nm (Liu et al., 2004), associated with deoxymyoglobin, metmyoglobin and oxymyoglobin, respectively (Liu & Chen, 2000). The intensities of these peaks changed significantly with storage temperature (Liu et al., 2004). Furthermore, two peaks at 1465 and 1960 exhibited a decrease in intensity with decreased freezing temperatures. The 1465 and 1960 bands can be attributed to the first overtone of OH–NH stretching, and a combination of OH stretching and deformation modes of water, respectively (Liu et al., 2004).

Whole bovine meat subject to three freeze (-18 °C) thaw cycles was directly analyzed and compared with fresh meat by visible/near infrared reflectance spectroscopy (Downey & Beauchene, 1997b). Factorial discriminant analysis (FDA) and soft independent modeling of class analogy (SIMCA) were applied to the visible-NIR data (650-1100 nm) before and after multiplicative scatter correction (Downey & Beauchene, 1997b). The major difference in reflectance between fresh and frozen meat spectra was observed at 762 nm (Downey & Beauchene, 1997b). The calibration model was performed with four clusters, fresh meat, meat frozen once, twice and three times. The factorial discriminant analysis after multiplicative scatter correction was most successful and identified none of the 48 thawed meat samples as fresh, but 3 out of 16 fresh meat samples were incorrectly identified as thawed once (Downey & Beauchene, 1997b).

Dried drip juice from whole bovine meat samples subject to three freeze (-18 °C) thaw cycles was analyzed by near infrared reflectance spectroscopy at wavelengths between 1100 and 2498 nm (Downey & Beauchene, 1997a). Both multiplicative scatter correction, FDA, SIMCA and partial least squares models were developed and applied to the spectral data (Downey & Beauchene,
The preferable factorial discriminant analysis involved six principal components and was applied to unmodified spectral data (Downey & Beauchene, 1997a). The analysis identified 3 out of 15 fresh meats incorrectly, but only one out of 46 thawed meats was wrongly identified (Downey & Beauchene, 1997a).

Ground meat from pork, chicken, and turkey was studied after storage at -30 °C by infrared spectroscopy (Al Rawder, Kemsley, & Wilson, 1997). A principal component analysis was performed on the data in the region 1000-1800 cm\(^{-1}\), and the individual species from fresh and thawed samples separated well. The actual results, which were only shown for chicken, demonstrated that out of 40 fresh and 40 thawed samples only one thawed sample was wrongly clustered in the group of fresh samples. Since the history of samples prior to purchase was unknown, it is possible that this one sample had actually been frozen.

Dry extract spectroscopy by infrared reflection (DESIR) (Gallo, Meurers, & Birth, 1990) was done on dried centrifuged beef juice from 120 samples equally distributed among fresh, frozen (-20 °C) and refrozen whole beef (Thyhold & Isaksso, 1997). Spectra were obtained from 400 to 2500 nm and principal component analysis was applied to all data (Thyhold & Isaksso, 1997). The k-nearest neighbor algorithm (k-NN) succeeded in 100% correct classification of 80 fresh and thawed samples. Thyhold and Isaksso (1997) also tested the ability to differentiate between fresh, frozen and refrozen meat, and 93% of the 80 beef samples were correctly classified. All above spectroscopic methods lack a full validation that includes parameters such as breed, muscle type and animal age. However, the results show that spectroscopy possesses a great potential in the analysis of both whole and ground meat.

Nuclear magnetic resonance

The transverse (T2) relaxation time is decreased significantly in thawed whole pork compared to fresh pork (Guilhenuef, Parker, Tessier, & Hall, 1997; Mortensen, Andersen, Engelsen, & Bertram, 2006), and the highest freezing temperature (-80 °C) resulted in the slowest T2 (Mortensen et al., 2006). The longitudinal (T1) was also studied and a similar decrease in thawed meat compared to fresh meat was observed for pork (Guilhenuef et al., 1997), beef and lamb (Evans, Nott, Kahriarsagar, & Hall, 1998). The magnetic transfer rate was investigated and increased in thawed pork (Guilhenuef et al., 1997), beef and lamb, when compared to fresh meat (Evans et al., 1998).

5. Bio imaging

It is well documented that slow freezing results in the formation of a few large extracellular ice crystals whereas rapid freezing produces many small ice crystals distributed throughout the meat tissue (Fennema, 1973; Martino et al., 1998; Sanz et al., 1999). The degree of damage caused by freezing is dependent on the crystal size and location but also recrystallization plays a major role. Recrystallization is lowered by decreasing the temperature, as shown by Huber and Stadelman (1970), who found that the lower the holding temperature of whole chicken the less the deterioration.

5.1. Microscopy

The size of ice crystals is dependent on the freezing condition and different sizes have been reported as the upper diameter limit. In beef an upper diameter size of 60 μm was reached after 30 days at -5 °C and the lower the temperature the longer the time needed to reach the upper diameter size (Martino & Zaratzky, 1988). Yet, another study where whole pork was air blast frozen (0.09-0.157 °C/min) and stored in a conventional freezer at -20 °C, showed irregular ice crystals with diameters of 93, 111 and 95 μm at the centre, midway and surface of the sample, respectively (Zhu, Bail, Ramaswamy, & Chapleau, 2004). They also showed that the crystal size was dependent on the freezing rate and the slower the freezing rate the larger the crystals.

The effects of the ice crystals formed were studied by the isothermal freezing substitution technique (Martino & Zaratzky, 1986) where samples are fixed in the frozen stage by Carnoy fluid. Studies on whole pork showed serious deformation and disruption of fiber bundles after air blast freezing, liquid immersion freezing and traditional freezing when compared to unfrozen pork (Martino et al., 1998; Molina-Garcia et al., 2004; Zhu et al., 2004). Temperature fluctuations have also been investigated and a study on whole beef showed greater deterioration in tissue submitted to either an increase or decrease in temperature during frozen storage, compared to that held a constant -20 °C (Martino & Zaratzky, 1988).

5.2. Electron microscopy

Carroll, Cavanaugh, and Rorer (1981) performed a storage experiment where thawed whole bovine meat was analyzed by scanning electron microscopy (SEM). Results from samples stored at -18 °C in still air for up to 26 weeks were similar throughout, except for a slight compaction of muscle fibers at week 26 (Carroll et al., 1981). These results differ from those of samples frozen in liquid nitrogen and then stored at -18 °C. These samples became progressively deteriorated and showed extreme compaction of muscle fiber and deep pitting of fibers with storage up to 26 weeks (Carroll et al., 1981). This is contradictory to the general idea that fast freezing produces smaller ice crystals that damage the meat to a lesser extent than slow freezing, which produces larger crystals.

Repeated freeze-thaw cycles at -10 °C and at -18 °C where performed on whole buffalo meat (Sen & Sharma,
One freeze-thaw cycle at −10 °C resulted in slight change that was not seriously increased on further freeze-thaw cycles but after freeze-thaw cycling at −18 °C, extensive damage of muscle fibers was observed.

Bovine meat subject to five freeze-thaw cycles was divided in two groups (Carroll et al., 1981). The first group was frozen for 24 hours at −18 °C and then thawed for 24 h at room temperature or at 2–3 °C. The other group was frozen 20.5 h at −18 °C and thawed at room temperature until the internal temperature reached 4 °C, a time of 3½ h. Drastic alterations in ultra structure were seen when the samples were allowed to thaw for 24 h at room temperature. Only a slight compaction of muscle fiber was seen when the thawing temperature was less than 4 °C.

It is concluded that freeze and thaw conditions have a major impact on the degree of microstructure deterioration and more research is needed to establish the exact relationship. Probably the major disadvantage in the microscopy discrimination of fresh vs. thawed meat is that under certain freeze-thaw conditions no obvious structural damage to the microstructure can be observed.

6. Sensory methods

Storage of meat unwrapped or wrapped in a vapor permeable material at subzero temperatures can result in freezer burns, which are caused by sublimation of ice from the surface. Freezer burns are easily detected by their opaque dehydrated appearance (Knapp & Weidemann, 1967) and are a clear indication that the sample has been poorly stored at subzero temperatures. A disadvantage in fresh vs. thawed meat discrimination by freezer burns is that they can be removed and visual analysis therefore misleading.

Another obvious visual feature is the freezing time dependent discoloration caused by a change in the hemoglobin environment (Deman, 1999) and studies on bone marrow and meat showed the red color decreased and a brown color increased after freezing, while little or no change occurred in refrigerated samples (MacDougall, 1982; Nicolalde, Stetzer, Tucker, McKeith, & Brewer, 2006). It is therefore possible to discriminate between fresh and thawed meat visually but also the odor and tenderness of meat is changed (Jakobsson & Tsson, 1973; Khan & Berg, 1967; MacDougall, 1982).

Early studies on rib steaks showed an increase in drip loss in thawed samples compared to fresh ones (Ramsbottum & Koonz, 1939) and a visible volumetric inspection can therefore discriminate fresh from thawed meat. A more recent study on fresh pork showed that the drip loss is 3.7 ± 2.2% of initial weight compared to 13.5 ± 2.3% for an air frozen 17 days stored samples (Hansen, Trinderup, Hvid, Daré, & Skibsted, 2003).

The sensory methods can act as a first line identifier of suspicious samples labeled as fresh meat and more objective advanced methods can subsequently be applied.

7. Discussion

7.1. Fresh vs. thawed meat authentication

All methods rely on detectable differences between fresh and thawed meat and a subsequent comparison. Most methods are capable of fresh vs. thawed meat discrimination in the case where fresh samples and thawed samples are taken from a common origin and analyzed at the same time under the same conditions. An unknown sample, analyzed for control purposes, seldom has a sample from a common origin for comparison and a method capable of discriminating fresh from thawed samples should therefore be based on a preceding large data set that can correlate or cluster detectable differences. A subsequent analysis of an unknown sample and the placement of results in the correct fresh or thawed meat cluster can then be done. Only the HADH enzymatic method (Gottesmann & Hamm, 1983) has clearly shown the possibility of discriminating between fresh and frozen (below −12 °C) thawed samples in a collaborative trial. Unfortunately, the method has a pitfall since prolonged or increased protease activity due to long term storage or inadequate freezing, can in theory digest the HADH and result in data that wrongly suggests the sample is fresh. Another pitfall is the possibilities that other freezing techniques could keep the mitochondria intact and therefore prevent HADH release and again result in data that wrongly indicates fresh meat. To overcome this problem one could use a second analytical method to verify the result. This additional method could be the Comet tail assay or microscopy, which could detect when meat has been frozen at temperatures from 0 to −12 °C and also the effects of long term storage. These additional methods should be validated and preferably subject to collaborative trials. The HADH method is considered to be the method of choice for discrimination between fresh and thawed meat as long as the whole meat sample has been frozen below −12 °C. The HADH method is not applicable to ground meat, because grinding releases HADH from the mitochondria, as is the case in frozen (−12 °C) thawed meat. In case of ground meat a spectrophotometric technique may hold promise.

7.2. Subzero storage time

If the length of frozen storage is to be determined in unknown samples a relationship between a molecular change and the time of storage must be known for that particular meat. In establishing frozen storage time it is crucial that the unknown samples have been treated identically to the samples used for comparison or used in a calibration. If the exact subzero storage conditions are known, it is possible to build a model that can determine the storage time. For this purpose a spectroscopic method would be a possible choice. Unfortunately, the history of most samples is unknown and establishing the time of storage therefore impossible with the techniques available today.
One theoretical alternative is to build a model based on a threshold limit that reflects a worst-case situation for a specific number of years. The subzero treatment of meat samples used to establish the threshold limit should involve freezing conditions that result in the largest molecular changes during a fixed period, e.g., two years. At subsequent analyses, unknown samples can either be below (low molecular change) or above (high molecular change) the threshold limit. A result below the threshold limit cannot be used to establish the subzero storage time, but a result above the threshold limit would indicate a subzero storage time exceeding two years. Whether this or other strategies in the determination of subzero storage time can be used in practice, is unknown.

References


