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Short Communication

Freshness assessment of stored meat by specific activity of glutathione transferase

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Abstract: We described a procedure for the determination of freshness of frozen meat or fish samples during storage periods. Correlations between decline in specific activities of glutathione transferase (GST) in fresh or frozen tissues of cow, chicken and fish with storage time at ambient temperature were examined. When fresh tissue is kept at ambient temperature, or if stored frozen and the sample is defrosted, leading to short or prolonged stay at ambient temperatures (20-30^{o}C), specific activity (y) of GST in the tissue begins to decline gradually with time (χ). We report that the relationship between the two parameters is of the form $y = -a\chi^2 - b\chi + c$; where a, b and c are constants and are dependent on the organ or tissue being examined. This relationship as exemplified by the quadratic equation is valid for all positive values of χ and y or when either is equal to zero, and permits the extrapolation of one parameter (χ, time of storage), if the other one (y, specific activity) is known. The results obtained also permit us to suggest that measurement of residual specific activity of GST in frozen meat or fish samples could provide information about storage history and, by extension, the freshness of the items.

Key words: frozen meat, fish, freshness, glutathione transferase, specific activity.

التقييم لجودة اللحوم المجمدة عن طريق قياس نشاط انزيم الجلوتاثيون ترانزفيراس

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الملخص : في هذا المقال العلمية تم وصف الاجراء المتبع لقياس جودة اللحوم المجمدة او الاسماك خلال فترة تخزينها. تم قياس معدلات هبوط نشاطات معينه لانزيم الجلوتاثيون ترانزفيراس GST في الانسجه المجمده او الحديثه للابقار والدجاج والاسماك في فترات تخزينها في درجه الحرارة المحيطه لها . عندما بقيت الانسجه الطازجة في درجات حرارة معينه او عند تذويبها بعد تجميدها لتظل في درجات حرارة الغرفه بين 20 و 30 درجه سيليزيه، لوحظ نشاط معين (y) لمادة GST في الانسجه حيث بدأت في الهبوط بالتدريج خلال فترات زمنيه محدده (X) .
وسجلنا العلاقه بينهما في المعادله التاليه $y = -a\chi^2 - b\chi + c$ حيث ان a ,b ,c هي ثوابت وغير مستقله عن النسيج الخاضع للتجربه. هذه العلاقه تمثل معادله تربيعيه وهي صحيحه لكل قيم x, y او عندما يكون أي منهما يساوي الصفر ، كما يقدر بقياس واحد براميتر عندما تكون x مده التخزين وتكون قيمه Y وهي النشاط المحدد معروفه.
النتائج التي حصلنا عليها تشير إلى ان قياسات النشاطات المحدده GST في اللحوم المجمده او عينات الاسماك قد توفر معلومات حول تاريخ التخزين وبالتالي مدى طازجة.

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Introduction

Determination of wholesomeness of frozen meat or fish for human consumption is important from health perspectives and several methods have been established to determine some of the quality parameters (Pearson, 1968; Swatland, 1995; Monin, 1998; Maltin et al., 2003).

The quality of frozen meat or fish sold especially in developed countries can be guaranteed to be relatively acceptable throughout the period of storage till final consumption because of stability of power supply which ensures that items stored under frozen conditions remain frozen for the intended period.

However, in many parts of developing countries, often, few sellers use satisfactory practice during handling of frozen foods (Gomaa et al., 2002). This is partly due to low education. Utility failure (electricity supply) may also be a contributory factor. For some countries that depend on importation of frozen meat, disruption in the cold chain in the course of transportation and subsequent storage are sometimes encountered, therefore compromising the wholesomeness of the frozen item. Items so compromised often get refrozen and sold to consumers. In addition to the health risk associated with such items, there is also the characteristic off-flavor formation which increases gradually with storage under the sub-optimum condition especially when diurnal ambient temperature is close to or above 25^{o}C as obtained in the tropical regions. Changes in the profile of cellular contents e.g. enzymes, proteins, fatty acids, amino acids, etc have been reported to be partly responsible for this.

Synthesis of proteins or enzymes in organs or tissues of vertebrates has been well documented in the last few decades, and both inducible and constitutive enzyme synthesis pathways are known to exist (Nelson and Cox, 2005).

The glutathione transferase (GST) we have chosen in this work as an index to measure meat freshness is usually present in high concentration in the liver of animals, performing house-keeping detoxication function, although some of the isoenzymes can also be induced (Sheehan et al., 2001; Dixon et al., 2002). In vertebrates, GST is also present in other organs namely: lung, kidney, skeletal muscle, intestine, heart etc., at different levels, performing partly the same function of detoxication. This enzyme which is ubiquitous and is predominantly expressed in the cytosol, is present to the same range in all tissues of the same species, in most cases.

While working on cytosolic GST from tissues of some animals (river prawn, fish-*Sarotherodon galilaeus*, cow), we have noted a progressive decline in specific activity of GST with time when such tissues are temporarily kept under ambient temperatures (25-30^{o}C). The decline in specific activity is probably due to internal redox reactions and proteolytic activity of endogenous proteases present in such tissues; which are triggered when the physiological control mechanisms of such tissues cease. It was reasoned that if such decline is progressive with time, determination of specific activity of a GST at any point in time, in a tissue, would give an indication of the length of exposure of such organ, and a reasonable conclusion could be drawn whether such organ, and by extension, other organs obtained from the same stock of animals are fresh enough, and fit for consumption.

We report here, for the first time, the possibility of using decline in enzyme activity to assess quality parameter. The method may be particularly important for freshness assessment after long transportation chains, and under some conditions of epileptic power supply leading to cycles of freezing-thawing and refreezing. GST is particularly useful for this study because of its presence in all tissues and also its ease of measurement.

Materials and Methods

Materials

Glutathione (GSH), 1-chloro-2,4-dintrobenzene (CDNB), bovine serum albumin (BSA) and Folin's reagent were products of Sigma Chemical Co. St. Louis, MO, USA. All other reagents used in this investigation were of analytical grade and were obtained from BDH Chemical Co. Ltd, Poole, England or other reputable commercial suppliers.

Fresh tissues of cow or chicken or fish were obtained locally. The animals were not administered with any chemical pretreatment before slaughter. Tissues from cow were obtained from animals that were reared on pasture in a free range system.

Methods

Preparation of crude GST extract

Approximately 500g of each tissue were cut into equal sizes and were left at room temperature (25-30^{o}C) to simulate the conditions usually prevalent in the local market where the carcasses are sold. At 4 or 6 hours intervals, samples were withdrawn and kept in the refrigerator at -20^{o}C prior to homogenization. Our laboratory had previously established that GST activity in fresh tissues remain relatively unchanged for several weeks of storage, when such tissues are stored at -20^{o}C or below (Adewale and Afolayan, 2005).

At the end of the exposure period, 30% homogenates of the different pieces, exposed to different lengths of time at room temperature were prepared in 10mM sodium phosphate buffer pH 7, 1mM EDTA, 1mM 2-mercaptoethanol, 10% glycerol. The 30% homogenate was centrifuged at 10,000g for 30 minutes at 4^{o}C. The resulting supernatant was filtered through cheese cloth to remove floating lipids and was used as the enzyme source.

Assay for GST and specific activity determination

GST activity was measured in a Cintra 101 GBC spectrophotometer following the method of Habig et al. (1974). A typical assay in a 3ml reaction mixture contained in the final concentration 1mM each of GSH and CDNB in 0.1M sodium phosphate buffer pH 6.5. An appropriate amount of enzyme was added to the reaction mixture to cause an absorbance change of between 0.02-0.05/min at a wavelength of 340nm. A unit of activity was defined as the amount of enzyme catalyzing the formation of 1μmole of the product –S-2,4-dinitrophenyl glutathione per minute taking extinction coefficient at 340nm to be 9.6mM^{-1}cm^{-1} (Habig et al., 1974). Specific activity was calculated as enzyme unit per mg of protein.

Protein concentration determination

The protein concentration of the extracts was determined according to the method of Lowry et al. (1951) using BSA as the standard protein.

Data analysis

Specific activities obtained for the extracts at different exposure times were plotted against their corresponding time (in days) using Microsoft Excel package. Curves and equations that best fitted the data points were also obtained using the same package.

Results and Discussion

Table 1(a) is a summary of the enzyme activity, protein concentration and specific activity against their corresponding exposure time typically obtained for cow liver. Table 1(b) and (c) are the summary for cow lung and chicken liver respectively. The specific activities (y) were plotted against exposure time (χ) using Excel package to derive a curve that best described the data generated as shown in Figures 1(a), (b) and (c) for cow liver, cow lung and chicken muscle respectively.

Their respective equations are also as indicated in the legend.

The curves are similar to one another and the equations of the curves are all quadratic in nature, and of the form: $y = -a\chi^2 - b\chi + c$. We attempted to establish whether the relationship between the measured specific activity (y), and time (χ) is linear, logarithmic, exponential or polynomial using Excel package. None produced the appropriate fitting for the data obtained, except the polynomial function. In the equation, a, b and c are constants; and the expression is valid for all positive values of χ and y, or when either is equal to zero. The constant terms are dependent on the organs under examination; absolute values of a and b are always greater than zero (Figure 1).

Table 1. Influence of exposure time on the specific activities of GST to different animal tissues. The values shown in the tables are averages of three independent determinations.

Exposure time (Hours)	Exposure time (in days)	GST activity (µmole/min)	Protein concentration (mg/ml)	Specific activity (units/mg protein)
(a) Tissues from cow liver				
0	0	12.63	44.2	0.286
6	0.25	11.69	46.1	0.254
12	0.50	12.31	51.4	0.239
18	0.75	9.31	44.3	0.210
24	1.00	6.44	43.7	0.147
30	1.25	4.5	40.7	0.111
(b) Tissues from cow lung				
0	0	2.075	22.84	0.091
12	0.50	1.44	18.51	0.0778
24	1.00	1.075	17.35	0.062
30	1.25	0.86	19.32	0.045
(c) Tissues from chicken muscle				
0	0	0.237	21.22	0.0111
12	0.5	0.252	23.73	0.0106
18	0.75	0.283	30.70	0.0092
24	1	0.178	23.65	0.0075
30	1.25	0.395	52.75	0.0075
36	1.5	0.245	40.58	0.0060

When the specific activity declines to zero, $-a\chi^2 - b\chi + c = 0$, which is similar to the ordinary quadratic equation $a\chi^2 + b\chi + c = 0$, and under this condition, $b^2 \geq 4ac$, for χ to be soluble, and this condition is always fulfilled unless the data generated are not good enough.

Thus knowing the specific activity (y) of a particular tissue sample can enable us to estimate the aggregate length of storage under inappropriate condition (χ) by interpolation from the graph or by solving for χ in the equation; which must have been prepared for that tissue at ambient condition, where the tissue would be exposed when compromised. For instance, in regions where the ambient temperature during a particular season is around 30°C, exposure would have to be done at this temperature, and standard curve prepared following the procedure earlier described. It should also be noted that the length of storage estimated for an unknown tissue under investigation is an aggregate of the total time the sample has been stored under inappropriate condition.

Examination of the tissues exposed to ambient temperatures at the end of twelve hours, revealed that decomposition has already started, and by the end of the exposure period, the tissues were no longer fit for consumption.

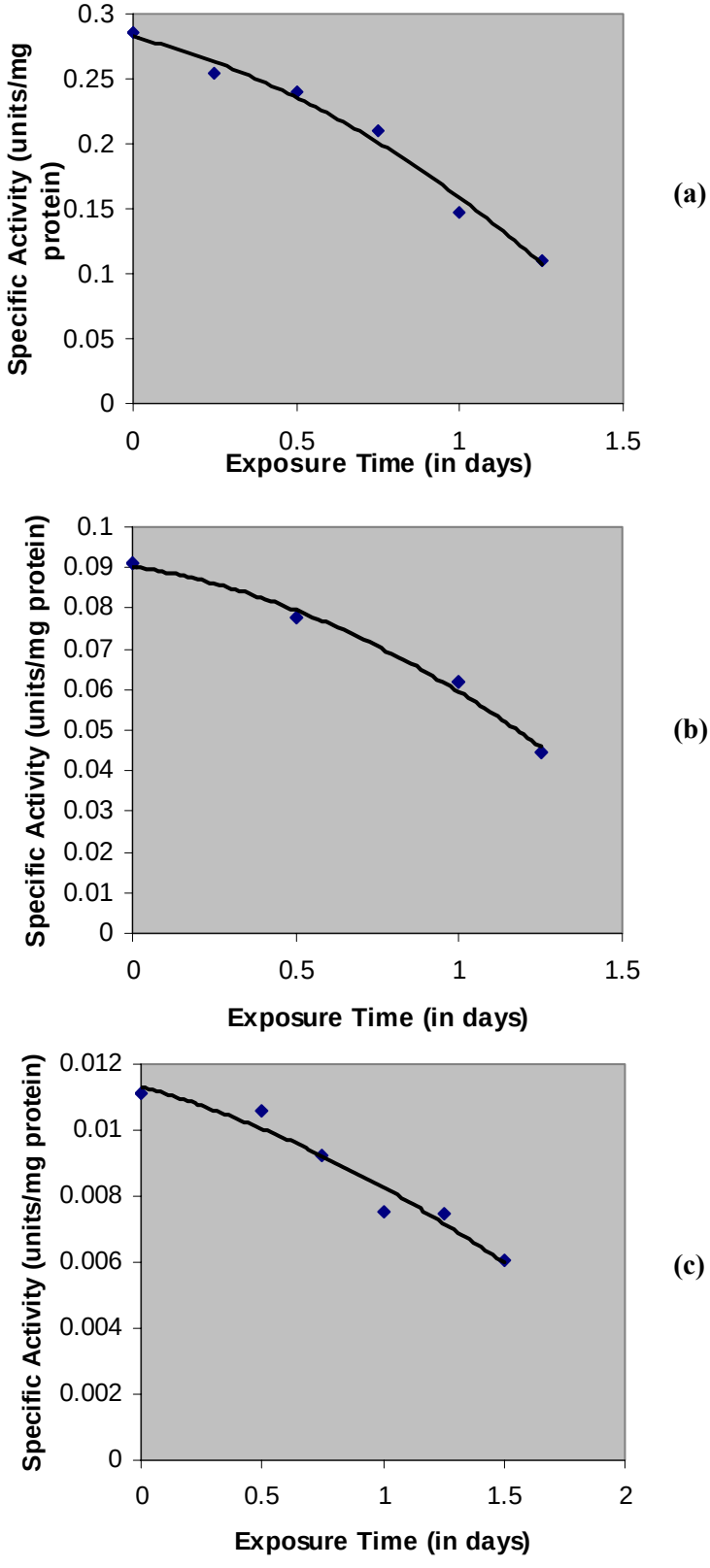

Figure 1. Plot of specific activities (y) against exposure time (χ) of tissues. Microsoft Excel was used to establish the curve that best fitted the data points. The equations were also similarly derived. A-profile obtained for the tissues from cow liver, while B and C are the profiles for cow lung and chicken muscle respectively. The equations of the curves are $y = -0.0623\chi^2 - 0.062\chi + 0.2823$; $y = -0.0185\,\chi^2 - 0.0124\chi + 0.0903$ and $y = -0.001\,\chi^2 - 0.002\chi + 0.0113$ for A, B and C respectively.

We have compared the method reported in this work with methods previously reported to assess meat freshness, by other workers, such as fluorescence method (Butov, 1967), use of potentiometric sensor (Kaneki et al., 2004), measurement of mixture of extractable volatile compounds of the gills in fish (Raatikainen et al., 2001), isolation or identification of some particular groups of spoilage organisms (Nychas et al., 1988; Yano et al., 2001) and the more recent, metal oxide sensor microarray (Musatov et al., 2010).

We are of the belief that some of these methods would necessarily require expensive equipment, which may be beyond the reach of would-be users, especially in developing countries, and this is partly why the method we have just described may be of interest.

In summary, we have presented a method for determining the storage history of frozen meat items, as a complement to other methods that are available. This technique can be useful in quality control and assurance to determine the freshness of the items. Our experience suggests that liver tissue is one of the best organs to use for the study. The technique is organ specific, implying that separate standard curves must be prepared for organs from the stock of the animals to be used. The procedure is straight forward, does not require expensive equipment and can be readily adapted to suit specific needs.

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