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Evaluation of beef aging by determination of hypoxanthine and xanthine contents: application of a xanthine sensor

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The changes of ATP-related compounds were measured during the aging of sirloin meat of eight bullocks, together with changes in myofibrillar fragmentation index (FI) and inosine-5-monophosphate (IMP) content. The increase in hypoxanthine (Hx) content, (Hx + 1/2 Xanthine (X)) content and *K* value correlated well with the observed increase in FI (R^2 = 0·804, 0·819 and 0·801, respectively) and decrease of IMP content (R^2 = 0·809, –0·839 and –0·965, respectively). An immobilized xanthine oxidase biosensor was tested for its usefulness as a convenient method for measurement of (Hx + 1/2X) content. Also, organoleptic evaluation and measurement of FI, free amino acids and ATP-related compounds were carried out. The findings suggested that the xanthine sensor could provide accurate measurements of the (Hx + 1/2X) content, and was useful for assessing the progress of aging by the estimation of the changes in tenderness and IMP content.

INTRODUCTION

The muscle of slaughtered cattle changes to a quality desirable for eating after a given length of aging (Smith *et al.*, 1978). The end-point of aging is determined by taking account of storage temperature and storage period. For objective judgment, several potential indices have been proposed and some convenient instruments to assess the progress of aging have been reported, such as the measurement of impedance (Pliquett *et al.*, 1990) and the measurement of K_0 value

K_0 (inosine(HxR) + hypoxanthine(Hx) + xanthine (X))/(ATP + ADP + AMP + inosine-5-monophosphate(IMP) + adenosine + HxR + Hx + X) × 100

(Nakatani *et al.*, 1986) which is a modification of the *K* value, where *K* = (HxR + Hx)/(ATP + ADP + AMP + IMP + HxR + Hx) × 100 (Saito *et al.*, 1959). For laboratory use, many other methods have been reported such as those using shear force value (Parrish *et al.*, 1977), fragmentation index of myofibrils (Olson *et al.*, 1976; Olson & Parrish, 1977), disappearance of troponin T and appearance of 30 kDa components in myofibrils by SDS polyacrylamide gel electrophoresis (Macbride & Parrish, 1977), extracted desmine content (Wismer-Pedersen & Weber, 1987) and sensitivity of Mg–Ca-enhanced ATPase activity in myofibrils (Ouali, 1983). However, as these methods are complicated and time-consuming, they were not suitable for routine use in quality control in meat packing factories.

Recently, biosensors consisting of a biological recognition system and a physicochemical transducer have been developed as new sensing instruments. Owing to their technical simplicity, rapidity and high selectivity, the use of biosensors has been introduced to the food industry (Turner *et al.*, 1987; Wagner & Guilbault, 1994).

The xanthine sensor was developed mainly for assessing the freshness of fish meat by measuring Hx using xanthine oxidase (Watanabe *et al.*, 1983; Suzuki *et al.*, 1989; Nguyen *et al.*, 1990). However, there have been no previous reports of studies in which the xanthine sensor was used to evaluate the freshness or the progress of aging in the meat of domestic animals and poultry.

As previously reported (Yano *et al.*, 1992), Hx is a useful index of aging. The major factor preventing the general application of *K* value or Hx as an index of aging, is that there is no direct relationship between ATP-related compounds and changes of tenderness and other organoleptic improvements during aging. However, as long as the sex and species of animals and portions of the meat are the same, and the ages of carcasses

are similar, it seems that ATP-related compounds can be used as an index of aging. As the changes occurring during aging are considered to be time-dependent, the time-lapse measured by Hx content is highly correlated with both tenderness and organoleptic improvement.

Contents of IMP (Sakaguchi *et al.*, 1991, 1992), free amino acids and peptides (Nishimura *et al.*, 1988) are important as taste substances in meat. The free amino acid and peptide contents increase during aging, whereas IMP decomposes to Hx via HxR, and the content of IMP decreases with time. Consequently, it is supposed that the measurement of IMP content is useful for estimating the palatability of meat, and IMP content can be estimated by Hx content.

Since three kinds of enzymes must be immobilized to measure IMP with biosensors (Okuma *et al.*, 1991), the maintenance of the sensor becomes complicated. Therefore, it is desirable to immobilize only one enzyme for the construction of an IMP sensor. When a xanthine sensor is constructed, only xanthine oxidase is required as an enzyme.

In the present study, the correlation between changes in tenderness and ATP-related compounds during storage of beef was confirmed, and the usefulness of the Hx or (Hx + X) contents was validated as aging indices. Also, the relationships between changes in IMP content and Hx or (Hx + X) contents during aging were demonstrated. After these initial experiments, the immobilized xanthine oxidase biosensor was applied to beef to determine its usefulness as a monitor of aging.

MATERIALS AND METHODS

Reagents

Xanthine oxidase (from butter milk) was obtained from Sigma (St. Louis, MO). All other chemicals were of analytical-reagent grade.

Sample preparation

For the preliminary experiments, sirloin meat was obtained from carcasses of eight 24-month-old Holstein bullocks stored at 0°C for 2 days after slaughter. The meat was cut into 25-mm-thick slices (weight: 250–300 g) and these slices were vacuum-packed in bags of high barrier film (Nylon/Binding layer/L.LDPE, 210 × 420 mm, 0·07 mm thick) and stored at 2°C. Viable counts, ATP-related compounds and fragmentation index were measured in two specimens of each sirloin meat sample.

For the application test of the xanthine sensor, two sirloin meat specimens were obtained from different Holstein bullocks. As well as monitoring by the xanthine sensor, the specimens were subjected to sensory evaluation and measurement of fragmentation index, Warner-Blatzler shear forces, and contents of free amino acids and ATP-related compounds. These specimens were stored as 50-mm-thick portions and cut in half to make 25-mm-thick slices before the experiments.

Viable bacteria counts

Bacteriological specimens were obtained by swabbing 25 cm^2 of the meat surface (5 cm × 5 cm) with sterilized cotton cloths, then homogenized with 100 ml of sterile 0·9% saline water. Decimal dilutions were spread over standard agar plates and colonies were counted after an incubation period of 10 days at 10°C. Duplicate experiments were performed for each specimen solution.

ATP and related compounds

ATP and its related compounds ADP, AMP, IP, HxR, Hx and X were determined according to the method of Yoshiura *et al.* (1986). Ten grams of the inner part of the meat was homogenized with 20 ml of 3% perchloric acid and centrifuged at 3000 rpm for 10 min. This procedure was repeated three times and the combined supernatant fraction was adjusted to pH 6·8. The precipitate formed was removed by centrifugation and the supernatant was made up to 50 ml with distilled water. Analysis was performed using a Shimadzu LC-6A high-performance liquid chromatography system with a reverse-phase Shimpack CLC-ODS (Shimadzu Co., Japan) column (6·0 × 150 cm).

The K value was then calculated according to the equation reported by Saito *et al.* (1959).

Amino acid content

The specimens prepared for determination of ATP-related compounds were also used for measurement of free amino acid content. Analysis was performed using a Jusco amino acid analysis system (LC-8000) with an ion exchange AApack Li^+ type (Jusco Co., Japan) column (6·0 mm × 100 mm).

Fragmentation index (FI)

Myofibrils were prepared from the inner part of the meat using the procedure reported by Yang *et al.* (1972), and the fragmentation index of myofibrils was measured according to the procedure described by Takahashi *et al.* (1967).

Sensory evaluation

The 25-mm-thick steaks were broiled at 200°C until the inner temperature reached 70°C, and were cut into 1 cm × 3 cm × 2 cm rectangular pieces. The warm specimens were served to a five-member trained sensory panel. The panel members evaluated the meat for tenderness, juiciness and flavor intensity on a five-point scale where 5 represented extremely tender, extremely juicy or very intensely flavored, and 1 represented extremely tough, extremely dry or bland.

Warner-Bratzler shear test

The part of the steak remaining after the sensory evaluation test was cooled to 25°C. Five cores (1·27 cm diameter) were removed parallel to the muscle fibers, and each core was sheared once with a Warner-Blatzler shearing device.

Xanthine sensor

Figure 1 shows a schematic diagram of the xanthine sensor used. This system consisted of a micro-tube pump, an auto-injector, a flow cell, an enzyme electrode, a thermostat, a system controller and a recorder. The enzyme electrode was based on the oxygen electrode in the end of which xanthine oxidase immobilized polymer membrane was fixed (Miyai & Asano, 1993). Oxygen consumption due to the oxidative activity of xanthine oxidase on X and/or Hx caused a decrease in the amount of dissolved oxygen around the membrane, and consequently brought about a marked decrease in the output current of the sensor. The current decrease between the initial stabilized output with the flowing buffer solution and the minimum output with the sample solution was measured for monitoring substrates. The thermostat was set at 30°C and the pH of the flowing buffer was 7·0.

The specimen solutions prepared for HPLC analysis were also analyzed using the xanthine sensor.

RESULTS AND DISCUSSION

Viable counts of bacteria

As the specimens were vacuum-packed and stored at 2°C, the viable counts increased only moderately. Even after 21 days, the viable counts were between 10^5/cm^2 and 10^6/cm^2, and no features of putrefaction were observed organoleptically. Therefore, the influence of bacterial growth on the values of FI and ATP-related compounds was considered negligible.

Changes in FI

Olson and co-workers (Olson *et al.*, 1976; Olson & Parrish, 1977) reported that FI was highly related to beef loin steak tenderness during aging. Thus FI was adopted as the fundamental value by which to monitor changes in tenderness in the present study. Figure 2 shows the changes observed in FI during storage. FI increased with time until around 15 days, after which time the rate of FI increase became slower and in some specimens ceased to change. At 0 day, the FI values were 29·2–41·9%, and these increased to 71·1–89·2% at 21 days. In two of eight meat blocks, the increase in FI stopped at 14 days, after which time FI values were maintained between 65·0 and 66·5%. From these observations, it was assumed that the rise in FI varied with the characteristics of the meat. Thus, the rate of aging was not the same in all specimens even though the species, sex, age, place of slaughter and chilling conditions were fixed.

As tenderness was not measured either organoleptically or instrumentally in the present study, it was not clear how FI value corresponded to the appropriate tenderness for consumption. However, according to Lawrie (1985), the appropriate aging period was about 10–14 days with storage at around 0°C. This period coincided with cessation of change in FI in the present study which occurred after 12–16 days of storage. Thus, the aging effect increases linearly until around 2 weeks, after which time the effect of aging on FI slows or stops. Therefore, the meat is suitable for consumption when this aging period has passed.

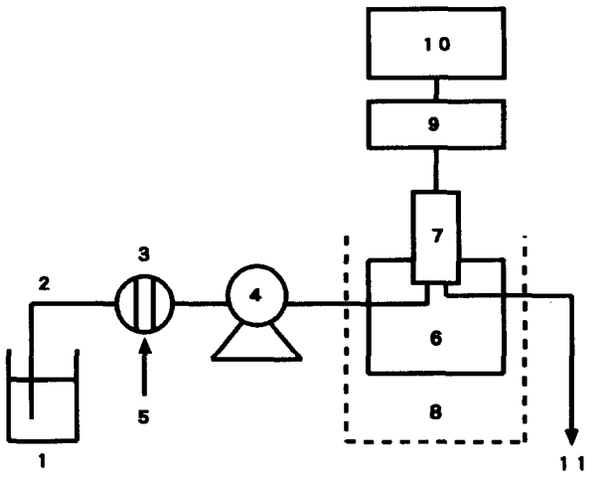

Fig. 1. Schematic diagram of the xanthine sensor: (1) carrier buffer (0·1M phosphate buffer, pH 7·0); (2) flow line; (3) auto-injector; (4) micro-tube pump; (5) specimen solution; (6) flow cell; (7) enzyme electrode; (8) thermostat; (9) system controller; (10) recorder; (11) waste.

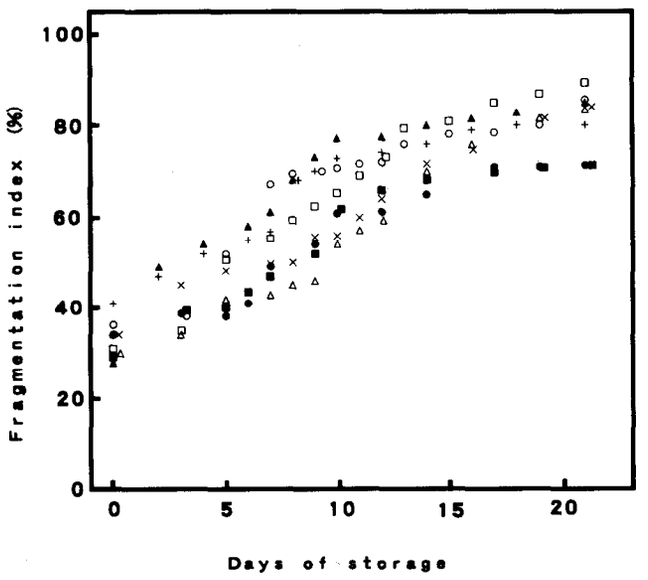

Fig. 2. Changes in fragmentation index during storage. Each of the symbols represents a different sirloin meat specimen. Eight blocks of meat were examined.

Changes in ATP-related compounds

After cattle are slaughtered, ATP in the muscle is degraded by the activity of intrinsic enzymes, with the resultant accumulation of HxR, Hx and X. Hence, the *K* value and Hx content have been proposed as indices of the progress of aging in meat (Nakatani *et al.*, 1986; Yano *et al.*, 1992).

The main object of the present study was to measure Hx contents using a xanthine sensor comprising immobilized xanthine oxidase. However, the activity of xanthine oxidase against X is half of that against Hx. Therefore, the values measured by such a xanthine sensor can be represented by the equation (Hx + 1/2X).

Figures 3–5 show changes in ATP-related compounds in meat with aging. The *K* value, Hx content and (Hx + 1/2X) contents showed linear increases until 21 days, and the rate of these increases differed between specimens similarly to those of FI. Since the carcasses were cut into portions 3 days after slaughter, ATP, ADP and AMP had already degraded and were not detected even on the first day of storage. Therefore, the *K* value in the present study was equivalent to the K_1 value [$(K_1 = (HxR + Hx) \times 100/(IMP + HxR + Hx)(\%))$] proposed by Karube *et al.* (1984) for the determination of fish freshness with multiple enzyme electrodes.

The *K* value had been proposed as a useful index of fish freshness and was also applicable to determine the freshness of chicken meat (Numata *et al.*, 1981). However, in beef which requires a long aging time, the *K* value indicates only time elapsed since slaughter. Therefore, the relationship between ATP-related compounds and fundamental aging indices such as tenderness must be determined.

As shown in Fig. 6, the IMP content decreased almost linearly from 4·91–6·24 μmol/g to 0·84–2·43 μmol/g. Although the day at which the IMP content is highest did not coincide with the most palatable day during storage of beef, IMP is supposed to be a fundamental component contributing greatly to the palatability of meat (Sakaguchi *et al.*, 1991, 1992). According to Sakaguchi *et al.* treatment of hot-water extracts prepared from aged beef with purified acid phophatase resulted in a marked lowering of flavor quality. Hence, the content of IMP remaining in aged beef is important for taste.

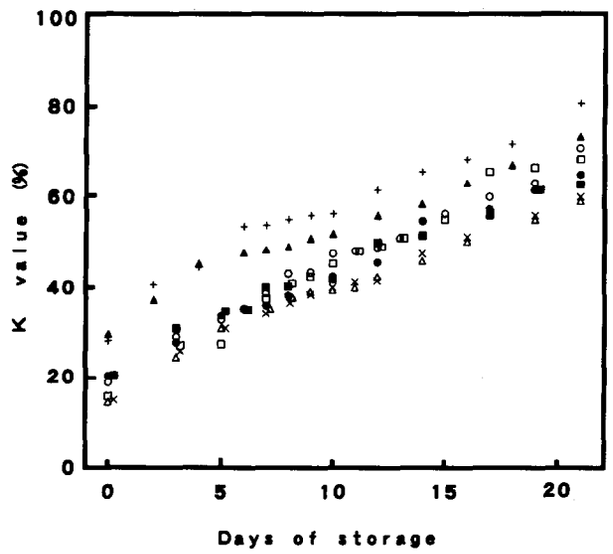

Fig. 3. Changes in the *K* value during storage. Specimens and symbols as in Fig. 2.

Regression analysis between FI and ATP-related compounds

As mentioned above, to utilize ATP-related compounds as an aging index the relationship between ATP-related

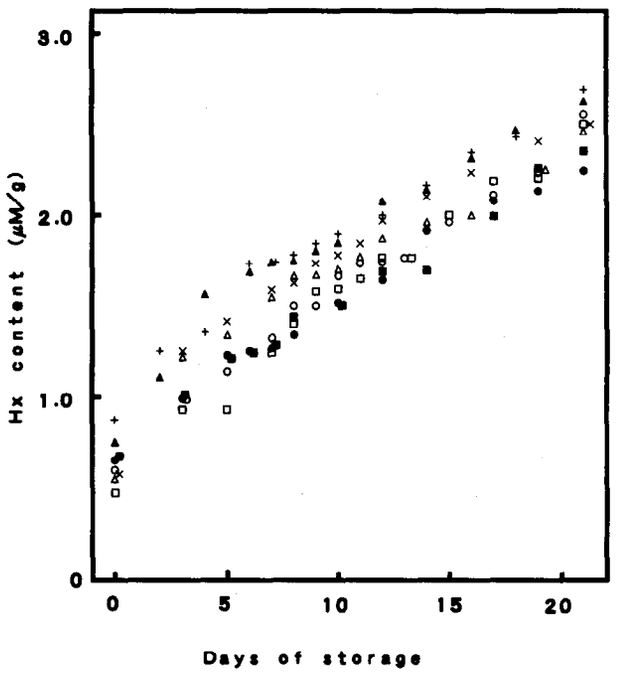

Fig. 4. Changes in hypoxanthine content during storage. Specimens and symbols as in Fig. 2.

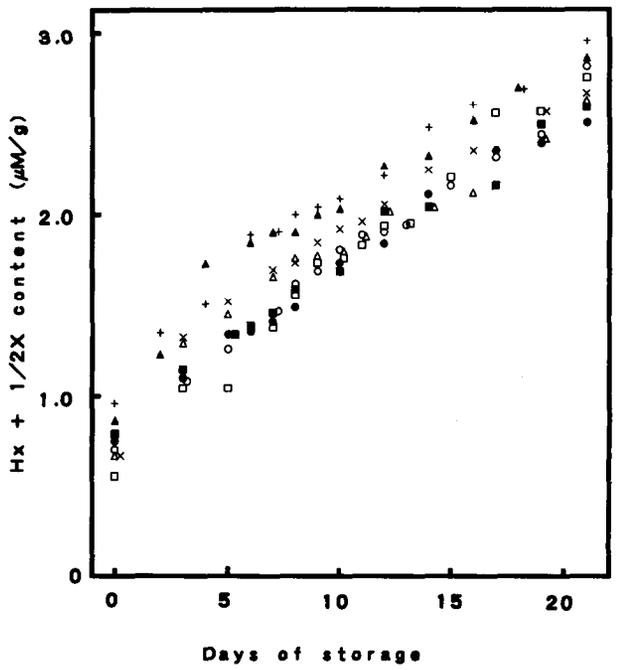

Fig. 5. Changes in the total amount of hypoxanthine and 1/2 xanthine during storage. Specimens and symbols as in Fig. 2.

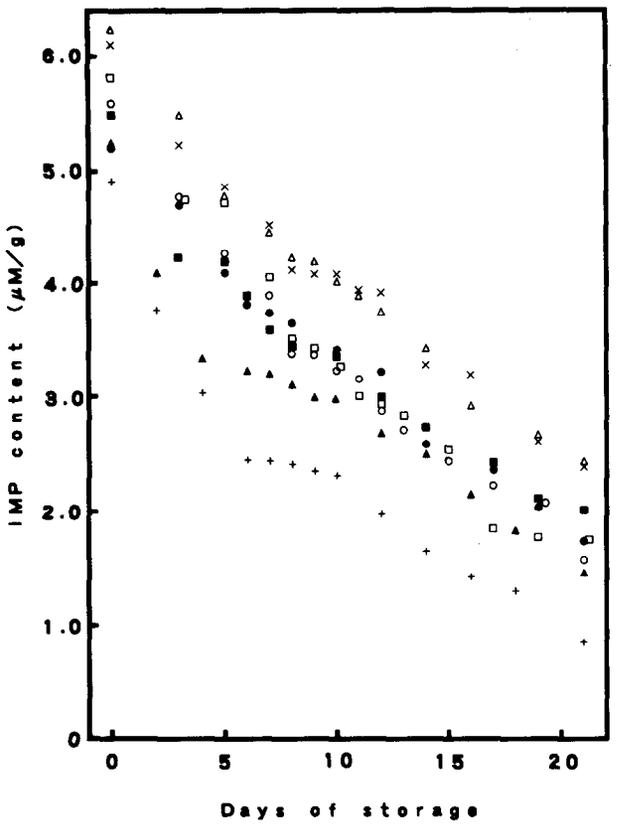

Fig. 6. Changes in IMP content during storage. Specimens and symbols as in Fig. 2.

compounds and tenderness must be determined. FI was adopted as a basic indicator of tenderness.

Table 1 shows the regression analysis between FI and *K* value, Hx content, and (Hx + 1/2X) content. The regression equations between FI and *K* value, Hx content and (Hx + 1/2X) content were $y = 1{\cdot}004x + 14{\cdot}915$, $y = 28{\cdot}463x + 13{\cdot}762$ and $y = 26{\cdot}622x + 12{\cdot}420$, respectively, and the correlation coefficients (R^2) were 0·801, 0·804 and 0·819 ($p > 0{\cdot}01$), respectively. These high R^2 values suggest that tenderness can be accurately estimated by measurement of ATP-related compounds.

Regression analysis between IMP and *K* value, Hx content and (Hx + 1/2X) content

As IMP decomposes and Hx accumulates via inosine, it may be possible to estimate IMP content by measurement of Hx content. Table 1 also shows the relationship between IMP content and *K* value, Hx content and (Hx + 1/2X) content; regression equations were $y = -0{\cdot}082x + 6{\cdot}983$, $y = -2{\cdot}029x + 6{\cdot}751$ and $y = -1{\cdot}9160x + 6{\cdot}880$, respectively, and the correlation coefficients (R^2) were 0·965, 0·809 and 0·839, respectively. The *K* value showed a high R^2 because the equation for calculating the *K* value contained IMP in its denominator. The R^2 values of Hx and (Hx + 1/2X) were considered to be high for estimating IMP content.

These findings suggest that changes in tenderness and IMP content of meat may be estimated by measurement of Hx or (Hx + 1/2X) content. We next applied the xanthine sensor to monitor beef aging, and its usefulness for this purpose was confirmed.

Application of the xanthine sensor to monitor beef aging

Before the application experiment, some fundamental properties of the xanthine sensor were investigated. The linear range was between 0·1 and 1·2 μmol/ml for Hx. Reproducibility was studied by analyzing the 0·5 μmol/ml Hx standard solution ($n = 10$), and the coefficient of variation was 0·86%.

Table 2 shows the changes in (Hx + 1/2X) content as determined with a xanthine sensor and in other indices during aging. In this experiment the progress rate of aging was higher than that in the fundamental experiment described above. The (Hx + 1/2X) content measured using the xanthine sensor and by HPLC increased linearly until 13 days along with FI. In contrast, the IMP content decreased linearly until 13 days. Shear force values showed increases in tenderness, but there was no clear point delineating cessation of the effects of aging. In the sensory analysis test, the scores of tenderness and flavor intensity increased until 8 days, and showed almost the same scores until 15 days.

The IMP content showed no correlation with the flavor score as determined by sensory analysis. The IMP content remained at 1·32 μmol/g in 8 days and it decreased to 0·92 μmol/g at 15 days. The threshold value of IMP was 0·47 μmol/ml in the sodium salt solution (Shimizu, 1971) and was in excess of this value even at 15 days. Therefore, it was supposed that no deterioration in taste occurred due to decomposition of IMP. However, over longer storage periods such as 1–2 months, taste might be affected by IMP contents because in such cases hardly any IMP remains.

The glutamic acid (Glu) and total free amino acid contents increased during storage. The threshold value of Glu was 1·60 μmol/ml in sodium salt solution (Shimizu,

Table 1. Correlation between FI or IMP content and *K* value, Hx content and (Hx + 1/2X) content

	FI		IMP	
	Regression equation	R^2	Regression equation	R^2
K value	$y = 1{\cdot}044x + 14{\cdot}915$	0·801*	$y = -0{\cdot}082x + 6{\cdot}983$	0·965*
Hx	$y = 28{\cdot}463x + 13{\cdot}762$	0·804*	$y = -2{\cdot}029x + 6{\cdot}751$	0·809*
(Hx + 1/2X)	$y = 26{\cdot}622x + 12{\cdot}420$	0·819*	$y = -1{\cdot}916x + 6{\cdot}880$	0·839*

*$P > 0{\cdot}01$.

Table 2. Changes in (Hx + 1/2X) content, IMP content, FI, shear force value, free amino acid content, glutamic acid content and sensory analysis in beef during storage at 2°C[a]

	Days							
	0	2	4	6	8	11	13	15
(Hx + 1/2X) (μmol/g)								
Xanthine sensor	1·07	1·42	1·60	2·15	3·33	3·39	3·41	3·46
HPLC	0·80	1·44	1·70	2·27	3·15	3·23	3·32	3·38
IMP (μmol/g)	4·77	4·20	3·47	2·31	1·42	1·23	0·97	0·92
FI (%)	26·1	30·6	43·0	59·3	64·3	66·0	74·9	76·1
Shear force value (kg)	3·53	2·47	2·18	2·03	1·98	1·77	1·83	1·66
Free amino acids (μg/g)	1665	2046	2118	2425	2711	2795	2910	2986
Glutamic acid (μmol/g)	0·19	0·37	0·42	0·83	0·92	0·99	1·11	1·19
Sensory analysis								
Tenderness	1·3	1·9	2·3	2·3	2·7	2·6	2·4	2·6
Flavor	1·7	1·9	2·1	2·1	2·6	2·6	2·4	2·5
Juiciness	1·9	1·8	1·8	2·1	2·3	2·2	2·1	2·2

[a]Means of two specimens.

1971) but was 1·17 μmol/g even after storage for 15 days. As already reported (Nishimura *et al.*, 1988; Sakaguchi, 1993), the palatability of meat cannot be explained merely by these two components — IMP and Glu.

The R^2 of (Hx + 1/2X) content between HPLC and xanthine sensor was 0·988 (n = 16, $P > 0·01$), indicating that the xanthine sensor was applicable for the measurement of (Hx + 1/2X) content in meat.

The R^2 between (Hx + 1/2X) content as determined by the sensor and IMP content or FI were 0·941 and 0·910 (n = 16, $P > 0·01$), respectively. Therefore, IMP content and FI could be accurately estimated by the xanthine sensor.

These findings suggest that, with application of the xanthine sensor, changes in tenderness and IMP content can be evaluated during beef aging. This sensor may therefore become a useful and conventional instrument for quality control of beef.

CONCLUSIONS

Changes in ATP-related compounds were measured during the aging of sirloin meat at 2°C, together with changes in FI and IMP content. Increases in Hx content, (Hx + 1/2X) content and K value correlated well with the increase in FI (R^2 = 0·804, 0·819 and 0·801, respectively) and the decrease in IMP content (R^2 = 0·809, 0·839 and 0·965, respectively). The immobilized xanthine oxidase biosensor was tested for its usefulness as a convenient method for the measurement of (Hx + 1/2X) content. Organoleptic evaluation and measurement of FI, free amino acids and ATP-related compounds were also carried out in the application study. The findings suggested that the xanthine sensor was capable of measuring the (Hx + 1/2X) content accurately, and was therefore useful for assessing the progress of aging by estimation of changes in tenderness and IMP content in meat.

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