ELSEVIER

0308-8146(94)00161-8

Food Chemistry 54 (1995) 155–159

0308-8146/95/$9.50

Changes in the concentration of biogenic amines and application of tyramine sensor during storage of beef

Yukio Yano, Nobuko Kataho, Mino Watanabe, Toyoo Nakamura

Central Research Institute of ITOHAM FOODS INC., 1-2 Kubogaoka, Moriya-machi, Kitasouma-gun, Ibaraki Pref., 302-01 Japan

&

Yasukazu Asano

Development Center of DKK Corporation, 4-13-14 Kichijoji Kitamachi, Musashino-shi, Tokyo 180, Japan

(Received 29 June 1994; revised version received and accepted 14 November 1994)

The vacuum-packaged beef was stored at 10, 5 and 0°C, and biogenic amines, viable counts and tenderness with the passage of time were measured. Of the biogenic amines analyzed, only tyramine was detected in viable cell counts in the order of $10^5 \sim 10^6$ before the appearance of a faint putrid smell (initial stage of putrefaction) at all three storage temperatures. Cadaverine was detected before the initial stage of putrefaction only at 5°C. The changes in tenderness ceased in 5 days (10°C), 10 days (5°C), 28 days (0°C), and the meat retained freshness judging from the viable counts and organoleptic evaluation. To estimate bacterial spoilage conventionally, a tyramine sensor which was composed of a tyramine oxidase-immobilized column and an oxygen electrode was applied. The sensor first detected tyramine at 5 days (10°C), 13 days (5°C) and 32 days (0°C). It was confirmed that the tyramine sensor was useful for estimating the bacterial spoilage in aging beef.

INTRODUCTION

Biogenic amines produced by bacteria have been studied from the viewpoint of the hazard caused by their psychoactive or vasoactive effect (Rice *et al.*, 1976) as well as their usefulness as indices of bacterial spoilage in food.

In the meat of livestock, the changes of biogenic amines during storage have been surveyed. Nakamura *et al.* (1979) reported a significant increase of putrescine (Put) and cadaverine (Cad) after the initial stage of putrefaction of pork meat. Slemr (1981) inoculated the spoilage bacteria dominant at low temperature storage of meat and stored aerobically at 5–8°C. Though the ratios of the formation of Put and Cad were different by the inoculated bacteria, these diamines increased significantly at the initial stage of spoilage. Wortberg and Woller (1982) analyzed the occurrence of biogenic amines in meat and meat products and showed that Put, Cad, histamine, tyramine (Tyn) were related to quality and freshness of meat and meat products.

According to Edwards *et al.* (1983) the significant changes in the content of Put and Cad did not occur until the meat was clearly spoiled. Conversely, Daher and Simard (1985) studied the correlation between bacterial counts and biogenic amines. Total and psychotrophic bacterial counts ranged from 10^2 to 10^9/g and were significantly correlated with Put, 1,3-diamino propane, Tyn, Cad and spermidine. They suggested that Put may be used as a direct bacterial count indicator in ground beef.

As Edwards *et al.* (1985) mentioned, the specimens were stored aerobically in those studies and there had been only few such studies of vacuum-packaged beef. Since bacterial flora vary with the environment in the package, the pattern of biogenic amine formation in vacuum-packaged meat differs from that in aerobically packaged meat. Dainty *et al.* (1986) have shown that Enterobacteriaceae, especially *Hafnia alvei* and *Serratia liquefaciens*, play a major role in the accumulation of diamines during storage of vacuum-packed meat at chill temperatures. While Cad does not require any metabolic input from other organisms, Put formation requires the growth of arginine-utilizing strains of lactic acid bacteria. The lactic acid bacteria which dominate the flora of stored vacuum-packed meats showed no

formation of Put and Cad. Consequently, when lactic acid bacteria become dominant in the micro flora from the early stage of storage, it is supposed that Put and Cad will be neither produced nor accumulated.

According to Edwards *et al.* (1987), Tyn may be a better indicator of spoilage/acceptability of vacuum-packaged beef because Tyn is formed by at least two of the major types of lactic acid bacteria which typically dominate the flora of vacuum-packaged meat. Smith *et al.* (1993) have shown that Tyn was detected after 20 days of storage and increased up to about 180 μg/g by 120 days of storage at 1°C in vacuum-packaged beef. Bacterial numbers increased until about 60 days when they levelled off at between 10^6 and 10^7/cm. In this experiment, the meat was organoleptically acceptable up to 60 days.

From these studies, Tyn seemed to be the best index of freshness in vacuum-packaged meat stored at chilled temperatures.

Currently over half of the total amount of the imported beef in Japan is transported by ship in vacuum packages in a chilled temperature range. By the time the imported beef reaches Japan, about 3 weeks has already passed after slaughtering. After customs clearance, the beef is further transported and stored until purchase by the consumer. Aging gives meat desirable qualities for eating, but too much storage for aging can lead to the growth of bacteria and the meat may become decomposed. Therefore, it is desirable to have a convenient method to estimate meat freshness especially in the long-term storage of meat at cold temperatures.

For the conventional determination of meat freshness Karube *et al.* (1980) developed a monoamine sensor consisting of a monoamine oxidase-immobilized membrane and an oxygen electrode. Using Put and Cad as an index, a putrescine sensor was developed consisting of a putrescine oxidase-immobilized membrane and an oxygen electrode (Yano *et al.*, 1988). Further, a tyramine sensor was developed to evaluate the quality of meat and fermented meat products (Yano, *et al.*, 1992). In this sensor, tyramine oxidase was immobilized on porous micro glass beads and filled into a small column.

The purpose of this study was to evaluate the usefulness of Tyn as an index of freshness during aging of vacuum-packaged beef and also of the applicability of the tyramine sensor.

MATERIALS AND METHODS

Sample preparation

Six sirloins (M. *longissimus dorsi*) of Holstein bullocks obtained from carcasses stored at 0°C for 2 days after slaughtering were used. The sirloin meats were cut into 40-mm-thick steaks. The cut specimens were vacuum-packed in bags made of high barrier film (Nylon/Binding layer/L.LDPE, 210 × 420 mm, 0·07-mm thick) and stored at 10, 5 and 0°C. The specimens were assigned to the experiments at proper intervals. The surface part of the meat was subjected to measurement of the bacterial count and biogenic amines. The remaining part was used to measure tenderness.

Measurement of bacterial counts

Ten grams of the surface part of the meat (5 × 5 cm) was shaved using a sterilized surgical knife and homogenized with 90 ml of sterile 0·9% saline solution. Decimal dilutions were spread over the plates of plate count agar (Eiken Chemical Co., Tokyo, Japan) for total aerobic viability counts. Colonies on the plate were counted after incubation for 10 days at 10°C for total aerobic viable counts. Duplicate experiments were made for each specimen.

Measurement of polyamines

Ten grams of the surface part of the meat (25 cm^2) was shaved and homogenized with 20 ml of 3% perchloric acid and centrifuged at 3000 × *g* for 10 min. This procedure was repeated three times. The combined supernatant was adjusted to pH 6·8 and the precipitate formed was removed by centrifugation. Distilled water was added to the supernatant to a volume of 50 ml. Purification and concentration were achieved for subsequent high-performance liquid chromatography (HPLC) analysis (Seiler & Deckardt, 1975; Seiler & Knodgen, 1979). The specimen was purified using an ion-exchange resin (Dowex 50w) column for eliminating amino acids. The polyamine fractions were dried with a rotary evaporator and the residue was redissolved in a small amount of perchloric acid. Tyramine (Tyn), putrescine (Put), cadaverine (Cad), spermidine (Spd) and spermine (Spn) were analyzed by HPLC on a reverse phase Shimpack CLC-ODS (Shimadzu Co., Kyoto, Japan) column (6·0 × 150 cm) equipped with a fluorescein detector, as reported by Gamou and Fujita (1987).

Measurement of shear-force value

The shear-force value was measured using a Rheoner (Yamaden, Tokyo, Japan) with a plunger attached to a stainless steel blade (0·2-mm thick, 37-mm wide, 8·0-mm long). The specimen was broiled at 200°C until the inner temperature reached 70°C and was then cut into 2 × 2·5 × 2 cm rectangles. Each specimen was sheared vertically to the meat grain by the plunger fitted to a 20-kg load cell. The crosshead moves with a speed of 1 mm/min/s until the 1-mm-clearance and the highest peak value was regarded as the shear-force value.

Sensory evaluation

The specimens were subjected to sensory evaluation just after the opening of the package. Five members of a trained panel sniffed the meat and within the day,

one of the members detected a faint putrid smell; this was regarded as the initial stage of putrefaction.

Construction of tyramine sensor

Tyramine oxidase was prepared by a modification of the procedure of Kumagai *et al.* (1969). *Micrococcus luteus* (IFO 12708) was cultivated in a medium which contained Tyn as the sole nitrogen source and tyramine oxidase was induced. The harvested cells were disrupted by an ultrasonic oscillator and purified to 5262 U/mg protein followed by ammonium sulfate fractionation, Sephacryl S-200 filtration, DEAE-cellulose column chromatography, hydroxyapatite column chromatography and a second Sephacryl S-200 filtration. The enzyme activity was measured colorimetrically with *o*-dianisidine and peroxidase. A unit of the enzyme activity was defined as the amount of enzyme which catalyzed the formation of 1·0 nmol of H_2O_2/min at 30°C.

The constitution of the tyramine sensor is the same as that previously reported (Yano *et al.*, 1992). This sensor consists of a manual injector, a peristaltic pump, an enzyme-immobilized column, a thermostat, a flow cell, an oxygen electrode, a detector and a microcomputer. Tyramine oxidase was immobilized on porous micro glass beads (CPG Inc. Fairfield, NJ, USA) with glutaraldehyde through Schiff base formation according to the method of Masoom and Townshend (1984). The enzyme-immobilized beads were put into a reactor column (50 × 3 mm i.d.) and flowed continuously until the beads were uniformly packed.

Oxygen consumption due to the oxidative activity of the enzyme immobilized in the column decreased the amount of oxygen dissolved in the flowing solution and consequently markedly decreased the output current of the oxygen electrode. The current decrease between the initial and the minimum currents was used for the determination of substrates.

The specimen solution which was neutralized and adjusted in volume for HPLC analysis was used for the measurement with the tyramine sensor.

RESULTS AND DISCUSSION

Changes in amine contents

The changes in the biogenic amine contents are shown in Figs. 1–3. Spn and Spd were detected from the initial day of storage and their contents remained constant at around 150 nmol/g and 10 nmol/g during storage, respectively. At all of the three storage temperatures, Tyn was detected on the days which Put and Cad were not yet detectable. At 10°C, Tyn, Cad and Put were first detected after 4 days (33 nmol/g), 5 days (5 nmol/g) and 7 days (5 nmol/g), and increased up to 272 nmol/g, 155 nmol/g and 76 nmol/g after 13 days, respectively. At 5°C, Tyn, Cad and Put were first detected after 10 days (7 nmol/g), 10 days (13 nmol/g) and 16 days (13 nmol/g), and increased up to 279 nmol/g, 89 nmol/g and 47 nmol/g in 22 days, respectively. At 0°C Tyn was first detected in 25 days (10 nmol/g) and increased up to 94 nmol/g in 39 days, but Cad and Put were not detected even after 39 days.

In mammalian tissues, Spn and Spd are present usually in significant (millimolar) concentrations. On the other hand, Put, which is a precursor of Spd, is usually

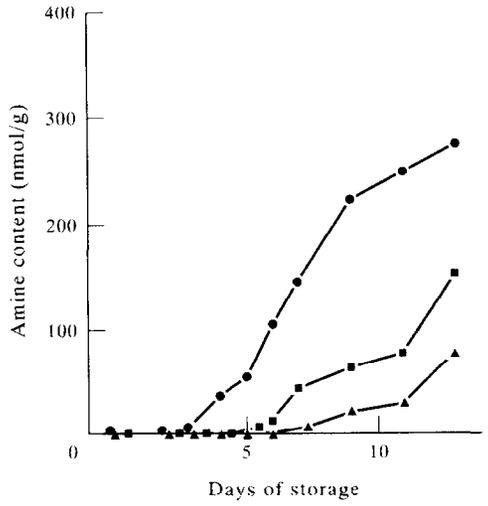

Fig. 1. Changes in the biogenic amine content during storage at 10°C. ●: Tyramine; ▲: putrescine; ■: cadaverine.

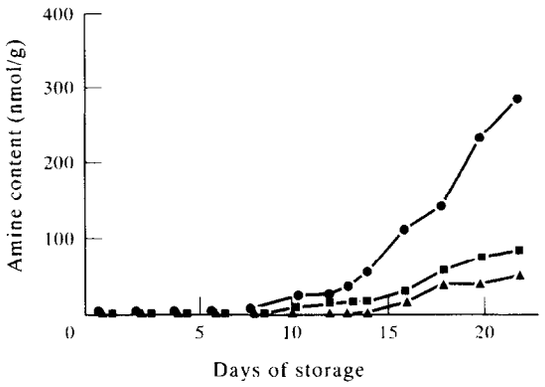

Fig. 2. Changes in the biogenic amine content during storage at 5°C. The symbols are the same as in Fig. 1.

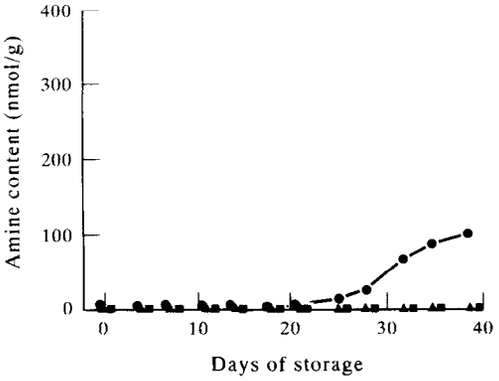

Fig. 3. Changes in the biogenic amine content during storage at 0°C. The symbols are the same as in Fig. 1.

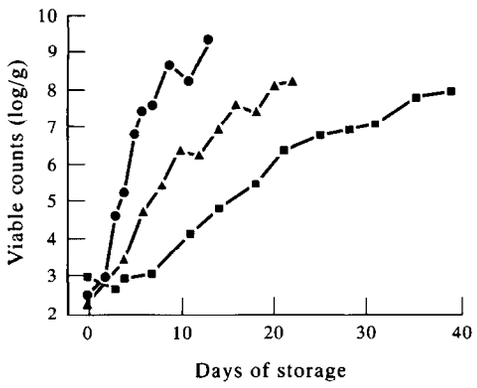

Fig. 4. Changes in the aerobic viable counts during storage. ●: 10°C; ▲: 5°C; ■: 0°C.

present in much lower (nanomolar) amounts and accumulates prior to the synthesis of Spd in tissues stimulated for increasing in size or cell number (Russell & Durie, 1978). Therefore, it was supposed that Put was not detected in the fresh meat by the HPLC method used in this study which had a detection limit of 5 nmol/g.

Relationship between the changes of viables counts, sensory evaluation and amine contents

The specimen gave off a faintly putrid smell in 6 days at 10°C, in 14 days at 5°C and in 35 days at 0°C. As shown in Fig.4, the corresponding viable counts were $2{\cdot}5 \times 10^7$ at 10°C, $8{\cdot}1 \times 10^6$ at 5°C and $4{\cdot}7 \times 10^7$ at 0°C, respectively and the amines which were detected were Tyn (99 nmol/g) and Cad (12 nmol/g) at 10°C, Tyn (52 nmol/g) and Cad (16 nmol/g) at 5°C and Tyn (83 nmol/g) at 0°C. Tyn and Cad were detected before the initial stage of putrefaction at 10 and 5°C, and only Tyn was detected at 0°C before that stage. Put was detected after the appearance of a faint putrid smell at 10 and 5°C.

The day of the appearance of faint putrid smell was set as the initial stage of putrefaction in this experiment and viable counts at that point reached the order of 10^7/g at 10°C and 0°C, and the order of 10^6/g at 5°C. Tyn was detected when the viable counts were in the order of 10^6 before the initial stage of putrefaction at all three storage temperatures. Therefore, Tyn can be used as an index for estimating bacterial spoilage in meat which still shows an acceptable state for eating. Tyn was superior to Cad as an index of bacterial spoilage.

Changes in tenderness

As expected, the tenderness decreased on storage (Fig. 5) and appeared to level off after about 3 days at 10°C, 10 days at 5°C, 28 days at 0°C, well before putrefaction was detected.

Application of tyramine sensor

From the above results, tyramine sensor appeared to be a useful tool for estimating bacterial spoilage. Before measurement of the specimens, the fundamental characteristics of the tyramine sensor were investigated and the results were almost the same as in the previous study (Yano *et al.*, 1992). The linear range of this sensor was from 10 nmol/ml to 800 nmol/ml when 200 μl of Tyn solution was injected. Substrate specificity was extremely high and only tryptamine showed about one-tenth of the activity of Tyn. The coefficients of variation were 2·8% in the standard solution and 3·5% in the meat specimen solution.

Figure 6 shows the changes of Tyn content measured by the tyramine sensor. Tyn was first detected in 5 days (65 nmol/g) at 10°C, 13 days (50 nmol/g) at 5°C and 32 days (71 nmol/g) at 0°C. The number of days until the first detection was delayed compared with HPLC analysis owing to the dilution and the detection limit, but were earlier than the initial stage of putrefaction at all three temperatures. After detection, Tyn content increased up to 268 nmol/g (13 days) at 10°C, 290 nmol/g (22 days) at 5°C and 109 nmol/g at 0°C with the same course as seen on HPLC. The values measured by the sensor coincided well with the HPLC values (R^2 =

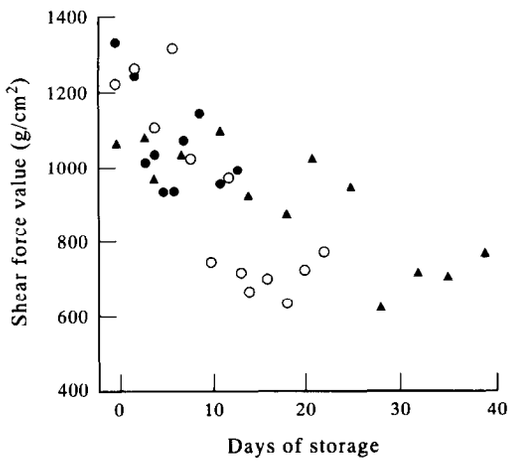

Fig. 5. Changes in the shear force value during storage. ●: 10°C; ○: 5°C; ▲: 0°C.

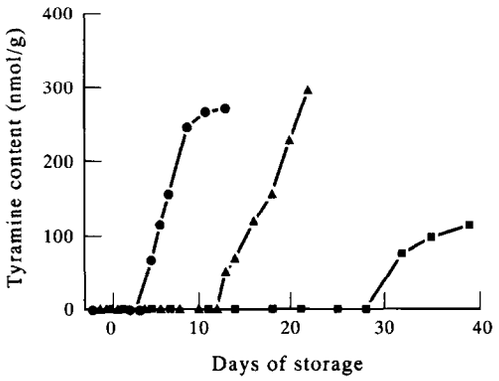

Fig. 6. Changes in tyramine content measured by tyramine sensor during storage. ●: 10°C; ▲: 5°C; ■: 0°C.

0·980, $\alpha > 0{\cdot}01$, $n = 20$.) Therefore, the tyramine sensor is useful for the evaluation of freshness in vacuum-packed beef.

Because Put and Cad can be used as an index for the bacterial putrefaction in some cases, the simultaneous application of tyramine sensor and putrescine sensor or the simultaneous immobilization of both enzymes will be effective for the evaluation of meat freshness.

The fundamental objective of this study was to detect biogenic amines produced by bacteria before the initial putrefaction. Karube *et al.* (1980) reported a monoamine oxidase electrode in which purified enzyme from *Aspergillus niger* was immobilized. As this immobilized enzyme had high activity toward various monoamines including Tyn, histamine and agmatine, the monoamine sensor could measure the biogenic monoamines non-specifically. Therefore, the monoamine sensor could accurately estimate meat freshness. Consequently, for evaluating the bacterial spoilage, the Tyn specific sensor is not necessarily needed. Besides, to evaluate meat freshness, the significance of the quantitative analysis in Tyn by tyramine sensor is to predict the risk of hypertension attacks for the patients being treated with a monoamine oxidase inhibitor. This is significant for the application of the tyramine sensor from the viewpoint of food hygiene.

CONCLUSIONS

Vacuum-packaged beef was stored at 10, 5 and 0°C, and biogenic amines, viable counts and tenderness were measured over time. Of the amines analyzed, only tyramine was detected in the order of $10^5 \sim 10^6$ viable cell counts before the appearance of a faint putrid smell (initial stage of putrefaction) at the three storage temperatures. Cad was detected before the initial stage of putrefaction at 10 and 5°C. For estimating the bacterial spoilage conventionally, the tyramine sensor, which was composed of a tyramine oxidase-immobilized column and an oxygen electrode was applied. It was confirmed that the tyramine sensor was useful for estimating bacterial spoilage in aging beef.

REFERENCES

Daher, N. S. & Simard, R. E. (1985). Putrefactive amine changes in relation to microbial counts of ground beef during storage. *J. Food Prot.*, **48**, 54–8.

Dainty, R. H., Edwards, R. A., Hibbard, C. M. & Ramantanis, S. V. (1986). Bacterial sources of putrescine and cadaverine in chill stored vacuum-packaged beef. *J. Appl. Bacteriol.*, **61**, 117–23.

Edwards, R. A., Dainty, R. H. & Hibbard, C. M. (1983). The relationship of bacterial numbers and types to diamine concentration in fresh and aerobically stored beef, pork and lamb. *J. Food Technol.*, **18**, 777–88.

Edwards, R. A., Dainty, R. H. & Hibbard, C. M. (1985). Putrescine and cadaverine formation in vacuum-packed beef. *J. Appl. Bacteriol.*, **58**, 13–19.

Edwards, R. A., Dainty, R. H., Hibbard, C. M. & Ramantanis, S. V. (1987). Amines in fresh beef of normal pH and the role of bacteria in changes in concentration observed during storage in vacuum packs at chill temperatures. *J. Appl. Bacteriol.*, **63**, 427–34.

Gamou, K. & Fujita, T. (1987) Determination of polyamines by reversed-phase high performance liquid chromatography. *Shimadzu Hyoron(Jpn).*, **44**, 55–9.

Karube, I., Satoh, I., Araki, Y., Suzuki, S. & Yamada, H. (1980). Monoamine oxidase electrode in freshness testing of meat. *Enymze Microb. Technol.*, **2**, 117–20.

Kumagai, H., Matsui, H, Ogata, K. & Yamada, H. (1969). Properties of crystalline tyramine oxidase from Sarcina Lutea. *Biochim. Biophys Acta.*, **171**, 1–8.

Masoom, M. & Townshend, A. (1984). Determination of glucose in blood by flow injection analysis and an immobilized glucose oxidase column. *Anal. Chim. Acta.*, **166**, 111–18.

Nakamura, M., Wada, Y., Sawaya, H. & Kawabata, T. (1979). Polyamine content in fresh and processed pork. *J. Food Sci.*, **44**, 515–23.

Rice, S. L., Eitenmiller, R. R. & Koehler, P. E. (1976). Biologically active amines in food: a review. *J. Milk Food Technol.*, **39**, 353–8.

Russell, D. H. & Durie, B. G. M. (1978). Polyamines as biochemical markers of normal and malignant growth. In *Progress in Cancer Research and Therapy*. (Vol. 8). Raven Press, New York, USA, pp. 3–4.

Seiler, N. & Deckardt, K. (1975). Determination of amines and amino acids in sugar-containing samples by dansylation. *J. Chromatogr.*, **107**, 227–9.

Seiler, N. & Knodgen, B. (1979). Determination of the naturally occurring monoacetyl derivatives of di- and polyamines. *J. Chromatogr.*, **164**, 155–68.

Slemr, J. (1981). Biogene Amine als potentieller chemischer Qualitatsindikator fur Fleisch. *Fleischwirtsch.*, **61**, 921–6.

Smith, J. S., Kenny, P. B., Kastner, C. L. & Moore, M. M. (1993). Biogenic amine formation in fresh vacuum-packaged beef during storage at 1°C for 120 days. *J. Food Prot.*, **56**, 497–500.

Wortberg, B. & Woller, R. (1982). Zur Qualitat und Frishe von Fleisch und Fleischwaren im Hinblick auf ihren Gehalt an biogenen Aminen. *Fleischwirtsch.*, **62**, 1457–63.

Yano, Y., Shibutani, Y., Hada, T., Nakamura, T., Miyai, J. & Ikeda, M. (1988). Estimation of meat freshness by polyamine sensor. In *80th Annual Meeting Abstracts of the Japanese Society of Zootechnical Science*, p. 98.

Yano, Y., Murayama, F., Kataho, N., Tachibana, M. & Nakamura, T. (1992). Evaluation of the quality of meat and fermented dairy and meat products by tyramine sensor. *Anim. Sci. Tecnol. (Jpn)*, **63**, 970–7.