Monitoring on Conditioning of Beef by Biosensors

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SUMMARY

For control of conditioning of beef we developed a biosensing system which can monitor both the initial stage of bacterial putrefaction and the progress of conditioning of beef. Our 2-line flow injection analysis (FIA) system was composed of autoinjector, microtube pump, oxygen electrode, water bath, system controller and recorder. Putrescine oxidase- and xanthine oxidase-immobilized membranes were fixed in two oxygen electrodes respectively. Vacuum packed sir loin meat of bullock was stored at 2℃ and 10℃. These samples were used for microbial and chemical analysis. In chemical analysis polyamines, ATP related compounds and fragmentation index were measured. Samples were also analyzed by 2-line FIA system and compared with the data by HPLC. In the intermediate temperature conditioning at 10℃, we could monitor the initial stage of putrefaction and the progress of conditioning by 2-line FIA system. However, in the low temperature conditioning at 2℃, putrescine sensor was not useful for detecting the bacterial putrefaction because lactic acid bacteria became the dominant microorganisms in the micro flora and they didn't produce putrescine and cadaverine.

INTRODUCTION

Recently, biosensors consisting of biological recognition system and a physico-chemical transducer have been developed and applied to many fields. In the food industry, enzyme electrodes for determining glucose, ethanol, lactic acid and other substances are practically used. For proper control of conditioning of beef we need to monitor the bacterial putrefaction and the progress of conditioning simultaniously. However, no device is available for simple and rapid analysis of these two indexes. The objective of our study is to develop 2-line flow injection analysis system using biosensors and apply this sensing system to conditioning process.

MATERIALS and METHODS

1. Samples

Sir loin meat from bullock carcass which were stored at 0℃ for 2 days after slautering were chopped 20mm thick. These samples vacuum packed in high barrier plastic bags and stored at 2℃ and 10℃.

2. Viable counts of bacteria

Viable counts of bacteria was determined by surface plate method. For counting aerobic bacteria, standard plate agar (Nissui) was used and incubated at 10℃ for 10 days. For counting lactic acid bacteria, APT agar (BBL) was used and incubated anaerobically at 25℃ for 5 days.

3. Polyamines

Putrescine, cadaverine, spermidine and spermine were analyzed with HPLC on a reverse phase Shimpack CLC-ODS (Shimadzu Co.) column (6.0×150mm) equipped with a fluorescine detector as reported by Gamou et al.(1987).

4. ATP related compounds

ATP, ADP, AMP, IMP, inosine, hypoxanthine and xanthine were analyzed with HPLC on a reverse phase Simpack CLC-ODS column as reported by Yoshiura et al.(1986).

5. Fragmentation index of myofibrils

Fragmentation index of myofibrils were measured according to the procedure described by Takahashi et al.(1967).

6. 2-line flow injection analysis system using biosensors

As illustrated in Fig.1 this system was composed of autoinjector, microtube pump, oxygen electrode, water bath, system controller and recorder. Putrescine oxidase- and xanthine oxidase-immobilized membranes were fixed in two oxygen electrodes. Fig.2 showed the appearance of this system.

RESULTS and DISCUSSION

1. Fundamental characteristics of 2-line FIA system

(1) Response curves

Typical response curves of putrescine sensor and xanthine sensor are shown in Fig.1. Oxygen consumption due to the oxydative activity of the enzyme caused decrease in dissolved oxygen around the membrane and consequently brought about the marked decrease in the output current of the sensor. The current decrease between the initial and the minimum currents measured for the determination of substrates. Fig.3 shows effect of flow rate on response curves. The response gradually increased with decreasing flow rate, whereas times for the raising of response curve and base line recovery were delayed. A flow rate of 1.0 ml/min gave a pertinent combination of the response intensity and analytical speed.

(2) Lineality

The calibration curves of two sensors are shown in Fig.4. Linear relationship was observed in the range of 10 to 100 ppm in putrescine sensor and 50 to 500 ppm in xanthine sensor.

(3) Reproducibility

Table 1 shows reproducibility of the sensors. Coefficient variations in standard solutions of putrescine and hypoxanthine were 1.73 % and 2.59 %, respectively. The values in sample solutions were 1.69 % for putrescine and 3.05 % for hypoxanthine.

2. Changes in microbial counts and chemical indexes during storage

Fig.5 and Fig.6 show changes in microbial counts and chemical indexes during storage at 10℃ and 2℃. Viable counts reached 10^7/g after 7 days at 10℃ and 21 days at 2℃. At 2℃ lactic acid bacteria was dominant in the micro flora. Putrescine and cadaverine were detected by HPLC after 7 days at 10℃ and putrescine sensor of 2-line FIA system could detect putrescine and cadaverine at the same time, whereas at 2℃ putrescine and cadaverine were not detected even after 21days. Generally conditioning of beef is accelerated when the storage temperature increases. Therefore, fragmentation index and concentration of ATP related compounds changed more rapidly at 10℃ than at 2℃. The concentration of hypoxanthine and xanthine increased linearly, which coincided well with the data by xanthine sensor. These results suggest that we can simultaneously monitor the initial stage of putrefaction and the progress of conditioning by this system at the intermediate temperature of 10℃. However, in the low temperature conditioning at 2℃ putrescine sensor was not useful for detecting the bacterial putrefaction because lactic acid bacteria became the dominant microorganisms in the micro flora and they didn't produce putrescine and cadaverine.

CONCLUSIONS

For monitoring conditioning of beef we developed 2-line FIA system using biosensors which could determine putrescine + cadaverine and hypoxanthine + xanthine. In the intermediate temperature conditioning at 10℃ and 20℃ we could simultaneously monitor the initial stage of putrefaction and the progress of conditioning by this system. However, in the low temperature conditioning at 2℃ putrescine sensor was not useful for detecting the bacterial putrefaction because lactic acid bacteria became the dominant microorganisms in the micro flora and they didn't produce putrescine and cadaverine.

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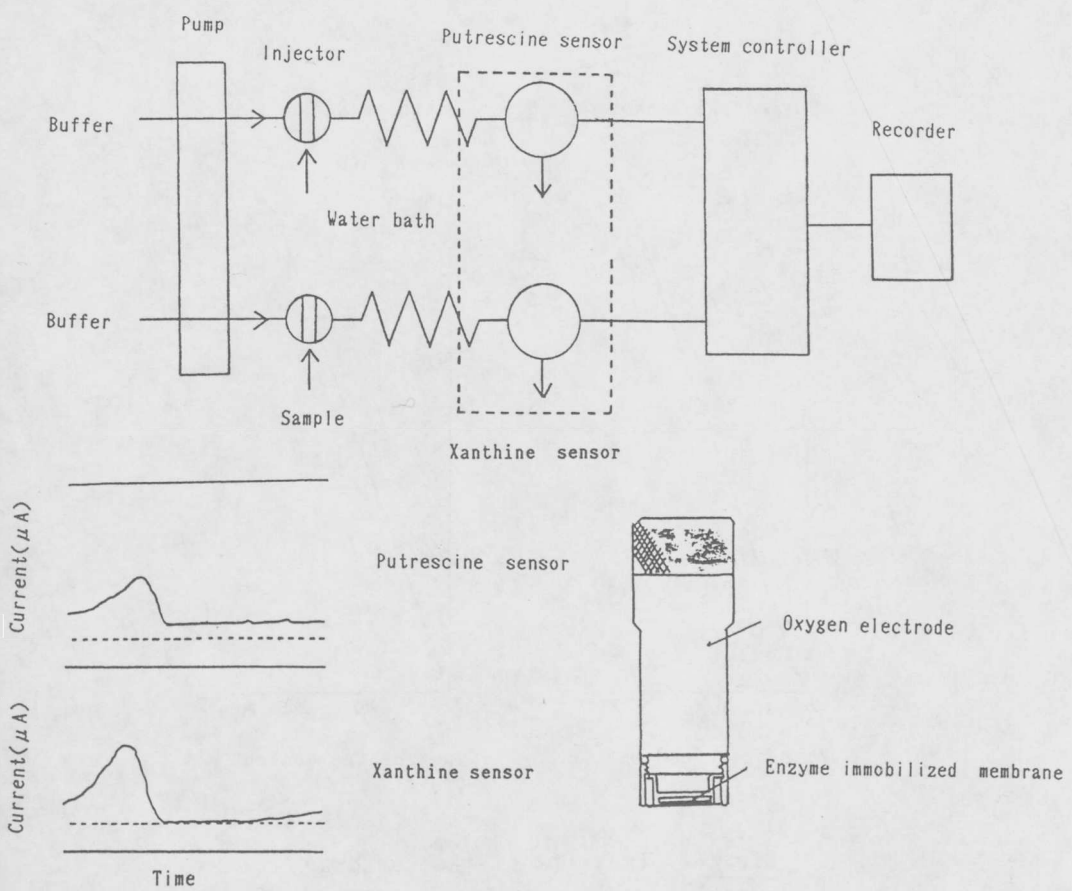

Fig.1 2-line FIA system using biosensors for monitoring on conditioning of meat

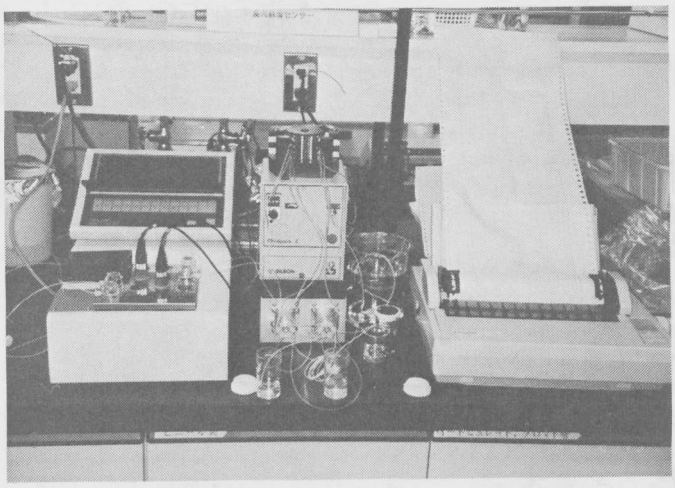

Fig. 2 Appearance of 2 line FIA system

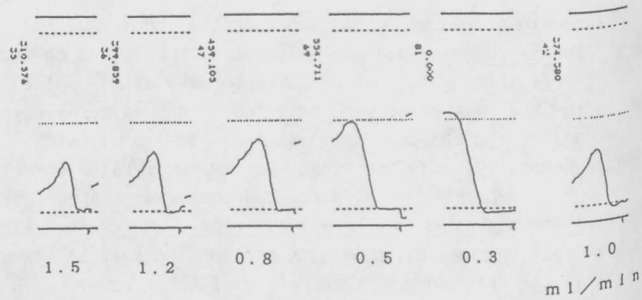

Fig.3 Effect of flow rate on response curves (Xanthine sensor)

Table 1 Reproducibility of the sensors

	Standard	Sample
Xanthine	2.59 %	3.05 %
Putrescine	1.73 %	1.67 %

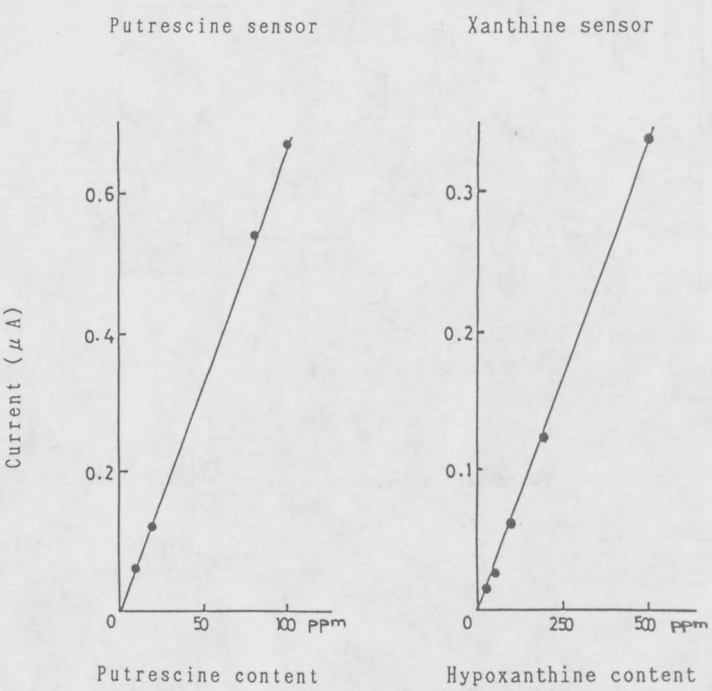

Fig. 4 Lineality of the sensors

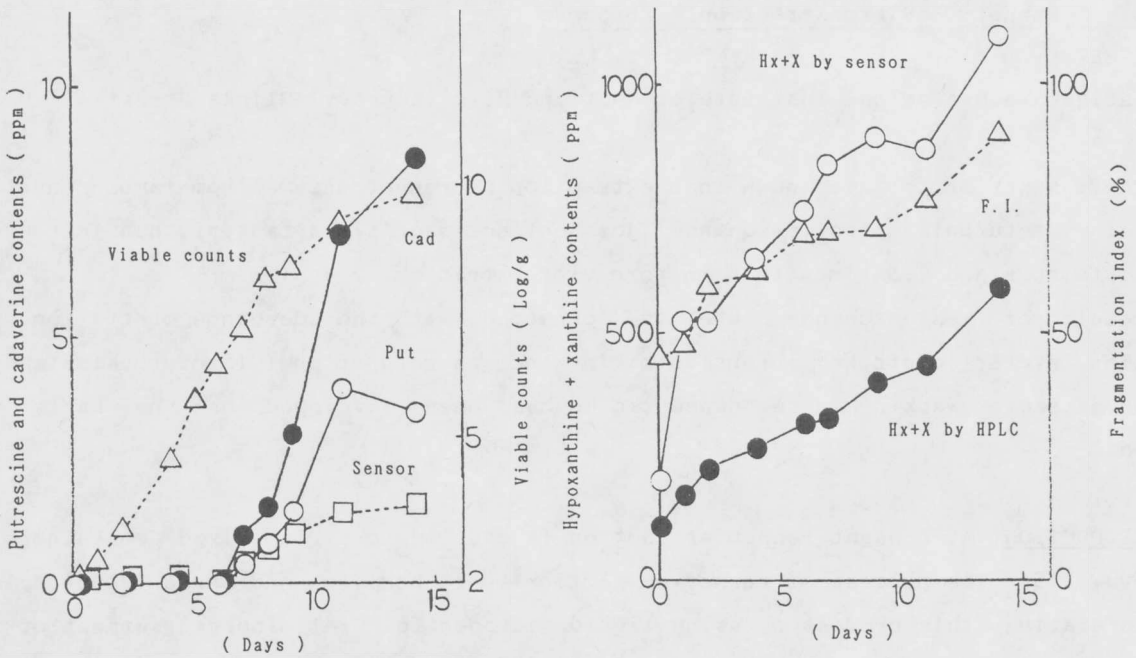

Fig. 5 Changes in viable counts, fragmentation index and concentrations of putrescine, cadaverine, hypoxanthine and xanthine stored at 10℃

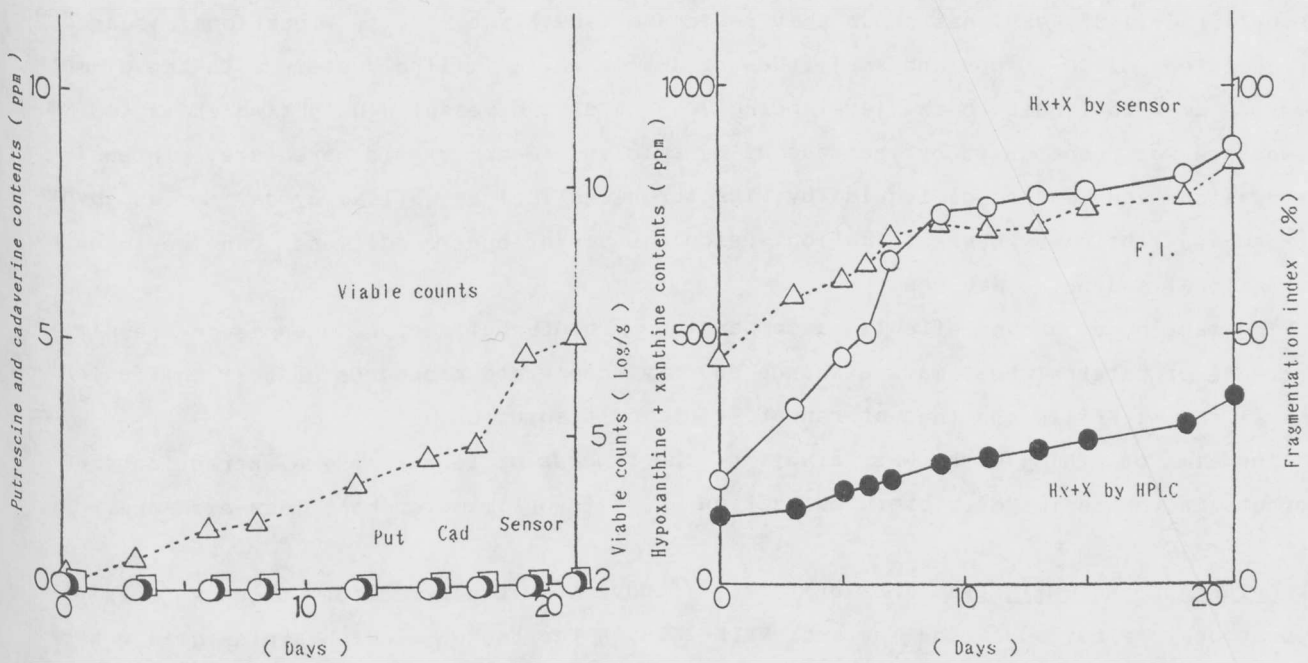

Fig. 6 Changes in viable counts, fragmentation index and concentrations of putrescine, cadaverine, hypoxanthine and xanthine stored at 2℃