# EVALUATION OF PORK HYGIENE QUALITY USING BIODETECTING METHODS

Ming Tsao Chen[1], Hsiu lan Kuo[2], Shu Mei Lin[2], Deng Lin[2] and Ming Ging Kuo[2]

1.Department of Bioindustry Technology, 112 Shan Jeau Rd., Da Tsuen, Changhua, Taiwan 515.
e-mail:michen@mail.dyu.edu.tw
2. Department of Food Sci. and Nutrition, Chung-Hwa College of Medical Technology, 89 Wen-Hwa 1 Alley, Jente Hsang, Tainan Sheng, Taiwan 717

## Background

Bacterial or other microbial contamination is the major cause of most incidents of food-borne illness. Among food groups, animal origin products are the most likely sources of pathogens threatening human health. To develop more accurate and non-time-consuming methods for evaluating hygiene quality of meat products is an important issue for securing day-to-day health of the consumers.

## Objectives

In the present study, we compare the activities of succinate dehydrogenase and hexosaminidase in raw meat samples collected from healthy animals (normal pork) or diseased animals (abnormal meat). We also utilize cultured murine macrophage cell line as biodetecting model for evaluating the microbe contamination in raw pork.

## Materials and Methods

Sample collection and preparation: Normal pork samples are obtained from the local meat market immediately after slaughter. Abnormal pork samples are collected from the animal died from illness in a local pig farm. Muscle proteins are extracted from *M. longissimus dorsi* according to the method reported previously by Kuo (1989), and used for enzyme activities and TNF measurements. For immune cell response assay, the extracts are added to the cell culture medium, and the cytokines released from the cell and cell viability are measured after the treatment. Microbiological assay: Microorganism growth is analyzed by FDA method. Immune response assay: Raw 264.7 cells, murine macrophages, are used for immune response assay. The cells are cultured in Dulbecco's modified essential medium (DMEM, GIBCO BRL, Rockville, MD) supplemented with 10 % feta bovine serum and incubated in an incubator at 37℃ with 5% $CO_2$. To determine the effects of muscle extracts on the immune cells, Raw 264.7 cells are treated with medium containing extract for 48 hours. Cytokine measurement: TNF in the medium is measured by enzyme-linked immunosorbent assay (ELISA) kit (Neogen). Cell viability: Cell viability after treated with muscle extract is determined by MTT assay. Succinate dehydrogenase (SDH) activity and Hexosaminidase activity: SDH activity is measured based on the procedure reported by Colowick *et al*. (1957). Hexosaminidase activity is assessed according to the method reported previously by Ching et al.( 2001).

## Results and Discussion

The data show that the level of volatile basic nitrogen (VBN), pH value and bacterial number are higher in abnormal pork than in the normal samples (Table 1); however, VBN concentrations in the early stage still remain at normal range. After 24-hour storage at room temperature, the level of VBN accumulation in the abnormal pork are higher than the normal pork but total bacterial counts of the abnormal pork are not higher than the standard values set by FDA, suggesting that total bacterial counts are not only an appropriate indicator for determining the quality of pork.

The results from enzyme activity assay demonstrate the alterations in the levels of cellular enzymes in the abnormal pork. The activity of HSA in abnormal tissue is much higher compared with the one in normal sample. The enzyme activity increases in the abnormal pork during storage, and reaches the level of absorbance 0.729 at 12 hour after storage, while the activity in the normal sample remains constantly at low level. The activity in the abnormal pork is 2 fold higher than the normal sample at every time point tested. The abnormal pork also exhibits significantly higher activity in SDH activity compared with the normal pork

at the early stage (within 6 hours) of storage. However, the activity in the abnormal pork decreases when storage period is prolonged. There is not significant difference in the activity of SDH between two groups of sample after 12 hour storage.

It is well known that microbial infection induces the production of TNF that can serve as an indicator for the infection. The abnormal pork tested in this study is obtained from the animal died from illness, and shows higher level of TNF compared with normal pork sample, indicating the previous infection before slaughter (Table 2). The level in the normal tissue does not change during storage. The TNF level in the abnormal pork is 2 folds higher than the level in the normal tissue at 12 hour after storage. The data from MTT assay further demonstrate the cytotoxicity of the extract from the abnormal pork in the cultured immune cells. Raw 264.7 cells treated with the extract of the abnormal pork shows lower cell viability after activation with LPS compared with the culture treated with the extract from the normal pork, suggesting the application of immune inhibition in Raw 264.7 cell as biodetecting model for evaluation of hygiene quality of pork.

## Conclusions

In conclusion, this preliminary study provides evidence for establishing new cellular indicators, including HSA activity, TNF production, and immune cell cytotoxicity, for discriminating abnormal pork from normal pork. This study also suggests viability of Raw 264.7 cells may be an appropriate indicator for biodetecting model for hygiene quality evaluation of pork. These parameters are quite stable within 24 hours, and are suitable for the hygiene quality evaluation in Taiwan. Since the traditional meat markets in Taiwan sell fresh raw meat only in the morning, within 6 hours after animal slaughter. Evaluating the hygiene quality of frozen and refrigerated products by detecting these parameters is an interesting subject for the future studies.

## References

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Table 1. Changes in freshness parameters and enzymatic activity of normal and abnormal pork during storage at 28℃.

| Parameters \ Storage time(hrs) | | 0 | 6 | 12 | 24 |
|---|---|---|---|---|---|
| pH | Normal | 6.13 | 5.98 | 5.77 | 5.98 |
| | Abnormal | 6.57 | 6.29 | 6.38 | 6.55 |
| Total bacterial counts (CFU/g) | Normal | 4.99 | 4.44 | 3.99 | 5.32 |
| | Abnormal | 4.51 | 5.42 | 5.83 | 6.55 |
| VBN (mg/100g) | Normal | 8.1 | 9.8 | 12.4 | 11.6 |
| | Abnormal | 20.8 | 19.7 | 37.6 | 51.0 |
| Succinate dehydrogenasse (Reduction rate %) | Normal | 21.3 | 31.0 | 36.9 | 22.7 |
| | Abnormal | 40.2 | 42.3 | 31.9 | 25.3 |
| Hexosaminidase (Absorbance at 400nm) | Normal | 0.289 | 0.258 | 0.255 | 0.238 |
| | Abnormal | 0.281 | 0.560 | 0.729 | 0.422 |

Table 2. Changes in TNF concentration and cytotoxicity of normal and abnormal pork during storage at 28℃ .

| Items \ Storage time(hrs) | | 0 | 12 | 24 |
|---|---|---|---|---|
| Tumor necrosis factor (TNF) concentration (pg/g) | Normal | 27.16 | 30.25 | 28.34 |
| | Abnormal | 34.55 | 58.69 | 38.38 |
| *Absorbance $_{at\ 570nm}$ of MTT cytotoxicity test by RAW 264.7 cells | Normal | 1.712 | 1.917 | 1.767 |
| | Abnormal | 1.947 | 2.064 | 1.927 |
| **Absorbance $_{at\ 570nm}$ of MTT cytotoxicity test by RAW 264.7 cells | Normal | 1.85 | 1.90 | 1.88 |
| | Abnormal | 1.69 | 1.58 | 1.52 |

MTT: 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazoilum bromide
*:Treatment without lipopolysaccharide ,**: treatment with lipopolysaccharide