POTENTIAL RAPID TEST OF ELECTRICAL CONDUCTIVITY (EC) RELATED TO MICROBIAL LOAD

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Abstract – Common measurements of commercial pork are pH, colour, electrical conductivity (EC), light scattering, and dielectric loss factor were used to distinguish Pale, Soft, Exudative (PSE) and Dark, Firm, Dry (DFD) pork. Interestingly, EC has been correlated with total viable and psychrotrophic counts in pork stored at 0 and 4°C. In order to develop field rapid test it is necessary to determine that pathogenic and spoilage bacterial concentrations are linearly correlated with EC using regression analysis. The spoilage and pathogenic bacteria in this study were *Pseudomonas fluorescens, Pseudomonas putida, Klebsiella pneumoniae* and *Staphylococcus aureus*. In terms of association of EC and microbial load, the correlation coefficients (*r*) of bacteria with EC in between 0.80-0.90 were highly significant. These results were well compatible with a previous study of linear correlation of EC and total plate count and psychrotrophic bacteria in pork. This study indicated that EC was correlated with level of spoilage and pathogenic bacteria contaminated in the pork. Further investigation is to monitor the increase of EC as a function of bacterial contamination level on pork surface simulating real pork storage for sale in the retail market. Then EC could be developed as rapid field test after determining the correlation of EC and level of spoilage bacterial loads of pork samples taken from retail market.

Key Words – Electrical conductivity, Microbial Rapid test

I. INTRODUCTION

Various techniques have been employed to differentiate Pale, Soft, Exudative (PSE) and Dark, Firm, Dry (DFD) from desirable quality of pork. Common measurements of commercial pork are pH, colour, electrical conductivity (EC), light scattering, and dielectric loss factor. The most conventional test is pH. This test directly reflects postmortem glycolysis of muscle and is also related to water-holding capacity, colour and meat-processing suitability. Meat colour is deemed a primary measurement for regular sensory test for pork manufacturers. [1]. Not only has EC been used as a tool to quantitatively rank pork quality[2] but also EC values has been used as an indicator of microbiological quality of pork[3]. Microbiologists applied impedance technique to follow microbial growth in the bacterial growth medium. The conductance increased as a result of charged metabolites generated by the microorganism from uncharged substrates using complicated equipment in laboratory[4]. Recently EC was correlated with total viable counts and psychrotrophic bacteria contaminated in pork stored at 0 and 4°C[3]. Furthermore, an apparatus measuring EC has been manufactured with an affordable price. This technology has made it possible to measure real-time EC of pork at the retail market to monitor changes of microbiological pork quality. Since EC of pork increased as a result of muscle fiber degradation during post mortem[2]. This phenomenon could confound the effect of microbial growth. It is initially and highly essential to establish pure correlation of EC and microbial growth *in vitro*. Therefore the objective of this study is to determine that pathogenic and spoilage

bacterial concentrations are linearly correlated with EC using regression analysis.

II. MATERIALS AND METHODS

Micro-organisms

Pseudomonas putida was provided by Department of Medical Science, Ministry of Public Health. *Pseudomonas fluorescens, Klebsiella pneumoniae* and *Staphylococcus aureus* were isolated from pork samples and identified by automated instrument VITEK® II system using GN and GP identification cards (bioMérieux Inc.). *Staphylococcus aureus* was also tested for coagulase production[5].

Bacterial suspension preparation

Pseudomonas spp. were cultured on nutrient agar and incubated at 25°C for 48 hr while *Klebsiella pneumoniae* and *Staphylococcus aureus* were cultured on nutrient agar and incubated at 37°C for 18-20 hr. The bacterial colonies were scooped and suspended in sterile normal saline solution to obtain a 0.5 McFarland (equivalent to 3.0×10^8 cfu/ml). Sterile normal saline solution was used to 5-fold serially diluted to obtain 50 ml bacterial suspension. This experiment were repeated 3 times.

Electrical conductivity measurement

The sensors for both conductivity and temperature were constantly submerged in bacterial suspension at a depth of 3 cm with a waterproof portable pH, conductivity, total dissolved solid (TDS), salinity, and temperature meter (PCSTestr35, Oakton® Instruments, IL. U.S.A.). The measurement range of EC was between 0-20 mS/cm. The temperature range of bacterial suspension was between 25-26°C.

Regression analysis

Independent (x) and dependent (y) variables were microbial load (cfu/ml) and EC (mS/cm), respectively. Linear regression was fitted to EC variable as a function of the microbial load variable using Microsoft® Office Excel. The goodness-of-fit (gof) of linear equation and degree of association of two variables were determined by sample coefficient of determination (r^2) and sample correlation coefficient (r), respectively.

Figure 1. portable conductivity meter and sensor

III. RESULTS AND DISCUSSION

The relationship between EC and all bacterial concentrations were statistically significant (p value < 0.001) as shown in Table 1. Whereas the goodness of fit of regression equation indicated that EC and microbial load are linearly correlated (Table 2). *Pseudomonas putida* had the highest r^2 and followed by *Klebsiella pneumoniae*. While *Pseudomonas fluorescens* and *Staphylococcus aureus* had lower r^2. Additionally, Gram negative bacteria e.g. *Klebsiella* spp. and *Pseudomonas* spp. seems to be more correlated with EC than Gram positive bacteria. In general, EC could be used as an indicator of either

spoilage or pathogenic bacteria contaminated in pork.

Table 1 Regression equations of EC (mS/cm) and microbial loads (cfu/ml)

Microorganisms	Regress equations
P. fluorescens	y = 0.106x + 13.57*
P. putida	y = 0.255x + 12.65*
K. pneumoniae	y = 0.214x + 12.91*
Coagulase positive *Staph.aureus*	y = 0.171x + 12.85*
Coagulase negative *Staph.aureus*	y = 0.189x + 12.74*

* statistically significant p value < 0.001

In terms of association of EC and microbial load, r of all tested bacteria with EC were highly significant (p value < 0.001). The correlation coefficients were also in line with slopes of regression equations (Table 1). These results were similar to a study of linear correlation of EC and total plate count (r = 0.91) and psychrotrophic bacteria (r = 0.84-0.89) in pork stored at 0 and 4^{o}C for 12 days and 22 days, respectively[3].

Table 2 Goodness-of-fit by linear regression and degree of association of EC and microbial loads

Microorganisms	r^2	r	p value
P. fluorescens	0.65	0.80	0.00030
P. putida	0.82	0.90	0.00032
K. pneumoniae	0.76	0.87	0.00002
Coagulase positive *Staph.aureus*	0.65	0.81	0.00028
Coagulase negative *Staph.aureus*	0.68	0.82	0.00016

In Germany, EC has been compared with different methods of pork quality evaluation e.g. pH value at 40 min postmortem (pH_{40}), pH value at 24 hr postmortem (pH_{24}), meat structure tester (MST), color (Göfo), and visual scoring. It turned out that EC at 40 min postmortem (EC_{40}) and EC at 24 hr postmortem (EC_{24}) were correlated well with all methods expect MST. Additionally, this study also recommended the EC threshold to classify meat quality at different times postmortem (Table 3)[2].

Table 3 Recommended EC threshold at different time postmortem

Meat quality	mS/cm	
	EC_{40}*	EC_{24}**
Good	< 5.0	< 8.5
Borderline	5.0-9.0	8.5-10.5
PSE	> 9	> 10.5

*EC_{40} : EC at 40 min postmortem
**EC_{24} : EC at 24 hr postmortem

Even though ultimate pH or pH_{24} was widely used to distinguish PSE or DFD pork. It is not possible to relate pH_{24} to microbiological quality of pork since pH is sensitive to acidic product from glycolysis resulting in PSE and DFD and a certain group of bacterial growth (particularly lactic acid bacteria which are minor initial spoilage bacterium). Therefore EC measurement could be a promising field rapid test to evaluate both textural and microbiological quality of pork.

Further investigations are to monitor the increase of EC as a function of bacterial contamination level on pork surface simulating real pork storage for sale in the retail market and to determine the correlation of EC and level of spoilage bacterial loads of pork samples taken from retail market. Additionally, differentiating PSE, DFD from good quality pork by EC measurement is also desirable tool for meat packers.

IV. CONCLUSION

This study indicated that EC was well correlated with level of spoilage and pathogenic bacteria contaminated in the pork. Then EC could be developed as rapid field test.

ACKNOWLEDGEMENTS

The full financial support was provided by the Thailand Research Fund (TRF).

REFERENCES

1. Chizzolini,R., Novelli,E., Badiani,A., Rosa,P., and Delbono,G.(1993).Objective Measurements of Pork Quality : Evaluation of Various Techniques. Meat Science 34 : 49-77.
2. Schmitten, F. Schepers, K.H. and Festerling, A. (1987). Evaluation of meat quality by measurement of electrical conductivity. In : P.V. Tarrant, G. Eikelenboom, and G. Monin, Evaluation and Control of Meat Quality in Pigs. (pp 191-200). Boston : Martinus Nijhoff Publishers.
3. Byun, J.S., Min, J.S., Kim, I. S., Kim, J.W., Chung, M.S. and Lee, M. (2003). Comparison of indicators of microbial quality of meat during aerobic cold storage. Journal of Food Protection 66 : 1733-1737
4. Gibson, D.M. (2001). Conductance/impedance techniques for microbial assay. In : E.Kress-Rogers and C.J.B. Brimelow, Instrumentation and sensor for the food industry. (pp 484-517). Cambridge: Woodhead Publishing Limited and CRC Press LLC.
5. Bacteriological analytical manual BAM (2001). *Staphylococcus aureus* : US.FDA. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071429.htm