The 5th Asian Conference on Lactic Acid Bacteria

Microbes in Disease Prevention & Treatment

1st – 3rd July 2009 Singapore

Organized by

NUS National University of Singapore

SSMB

Under the auspices of

AFSLAB

Main Sponsor

益多 Yakult

Co-sponsor

Abbott A Promise for Life

DANISCO First you add knowledge...

Supported by

Nestlé Nutrition INSTITUTE

S6
FUNCTIONALITY POTENTIAL OF "PETIS" AN INDONESIAN FERMENTED MEAT WITH *PEDIOCOCCUS ACIDILACTICII* YDA3 AND *PEDIOCOCCUS PENTOSUS* YDA4 STARTER CULTURES

Yoyok Budi Pramono*, Endang S. Rahayu, Suparmo**, and Tyas Utami****
* *Faculty of Animal Science, Diponegoro University, Indonesia*
Post Graduate Student of Food Science, Faculty of Agricultural Technology, Gadjah Mada University, Indonesia
Email: yok_b_p@yahoo.com
** *Faculty of Agricultural Technology, Gadjah Mada University, Indonesia*

Petis, an Indonesian Fermented meat is *pasta-like* product used as side dish traditionally fermented spontaneously. The study was design to introduce potential locally isolated lactic acid bacteria to improve its organoleptic and functional properties. The functional properties studied were ability to inhibit *coliform* and histamine producer bacteria, flavor improvement, and enhancement of *carnosine* production. *Carnosine* is bioactive peptide ß-alanyl-L-Histidine capable in maintaining physiologic performance and reduces the tendency of development of degenerative diseases.

Result indicated that the application of each starters reduces the *coliform* and histamine producer bacteria by 1 *log cycle* compared to spontaneous fermentation as control. Soluble protein increase by 10.3 %, reduce histamine by 5.8 mg/100g, suppressed of Total Volatile Based Nitrogen (TVN) by 42.8 mg/100g and Tri Methyl Amine (TMA) by 23.2 mg/100g. Enhance *carnosine* production by 3.16 times and 2.92 times, respectively. It showed that both isolates capable in enhancement of product quality, safety, and functionality.

Keywords: Meat fermentation, *Pediococcus*, functional property, *carnosine*

INTRODUCTION

The role of lactic acid bacteria is essential, especially in minimize on the undesired growth of bacteria, that is bacteria that cause the rottenness and pathogen bacteria. Functional starter culture offer an additional functionality compared to classical starter cultures and represent a way of improving and optimizing meat fermentation process and achieving tastier, safer, and healthtier products (De Vuyst, *et al.* 2006)

The bacteria that cause rottenness in the products of fermentation food will cause the formation of "off-flavor" components which is less favored. That is why, it is effort to minimize the formation of component indicators of "off-flavor" because those are influential towards the quality of fermentation products. The components of "off-flavor" are coming from the volatile compounds that are produced as the beginning of indication of rottenness from the continual degradation (over degradation) of amino acid or peptide. The parameter that is often used is TVN (total volatile base nitrogen) and formed TMA (tri methyl amen). That is cause, the fermentation of *petis* of meat generally is potential to form the components which are able to disturb the quality of health, so it is needed the control action by using the lactic acid bacteria as the starter cultures. Mayyo and Ammor (2007) was show that lactic acid bacteria have long been used as starter cultures in the production of fermented dry sausages and other meat-derived commodities. These cultures are generally designed to meet food safety, shelf-life, technological effecticeness and economic feasibility criteria.

The production of fermented meat was based on the use of starter cultures, for instance lactic acid bacteria that initiate rapid acidification of the raw material. Recently, new starter cultures of lactic acid bacteria with an functionality are being developed. The latter can contribute to the microbial safety or offer one or more organoleptic, technological, nutritional, or health advantages (Leroy and De Vuyst, 2004).

Technology aspects as related to microorganisms in functional food were highly complex and diverse. Processing of microbial systems for functional foods was dependent on the composition and processing history of the raw material used as

substrate, the viability and productivity of the starter cultures applied and the processing and storage conditions of the final food products (Knorr, 1998).

To improve functionality and add nutritional value to meat fermentation, its muscle protein was hydrolyzed by the proteases from lactic acid bacteria or indigenous enzymes from raw materials (Yin *et al.*, 2005). The purpose of this research is to reveal the role of bacteria of *Pediococcus acidilacticii* YDA3 and *Pediococdus pentosus* YDA4 in the fermentation of *petis* of meat. This fermentation is supposed to control the undesired components and the bacteria that will be the cause.

Materials and Method

This research was exploration research ("exploration experimental method") with 4 repetitions and 2 sub-units of experiment. There were 2 isolates of lactic acid bacteria which were used. Those were *Pediococcus acidilacticii* YDA3 and *Pediococcus pentosus* YDA4. These isolates were the results of previous research of meat spontaneously fermentation.

The products of meat fermentation were made by milled and salted meat with the solution of 20% (b/b) and the inoculated with starters. Then, it was done the refrigeration on the temperature of 4°C during ± 6 hours. The fermentation was running on the room temperature during 48 hours. The observed parameter was the ability to minimize on the group of *coliform*, the producer bacteria of histamine, TVN and TMA as the component indicators of "off-flavor" and several chemistry indicators, such as dissolved proteins, titrated total of acid, produced histamine and the changes of pH. It was used with selective media of VRBA (Violet Bile Red Agar) to get the group of *coliform*. Meanwhile, it was used with the modified Niven media to get the producer of histamine. It was used titration method to measure TVN and TMA. Meanwhile, it was used Lowry's method for the dissolved proteins.

Results and Discussion

1. The influence of Starter Cultures towards The Microbiological Change

The fermentation of *petis* of meat used 2 chosen isolates. Those were *Pediococcus aciddactich* YDA3 and *Pediococcus pentosus* YDA4. These both of isolates influenced the change of population of fermentation microbe of *petis.* The data of this research about the influence of isolates of *Pediococcus acidilacticii* YDA3 and *Pediococcus pentosus* YDA4 towards the change of population microbe will be presented in Table 1.

It is seen that both of isolates lactic acid bacteria, *Pediococcus acidilacticii* YDA3 and *Pediococcus pentosus* YDA4 have the ability to minimize the growth of group of *coliform* and the histamine producer bacteria up to 1 log cycle if those are compared with the control. The ability to minimize the group of *coliform* and the histamine procedure bacteria is guessed to come from the formation of lactic acid with its products metabolic. The products metabolic are peroxide hydrogen, *diasetil*, and bacteriocin. The lactic acid will diffuse into the cell of microbe which will dissociate, so it will disturb the transport system of nutrition. The dissociation to this cell will form the proton and anion, so that the existence of proton will disturb the balance of nutrition transportation. That was cause the bacteria will try to take the proton outside from its cell.

Table 1. The Change of Microbiological Population of Meat Fermentation - *Petis* - in Salt Solution of 20% (b/b) by Using 2 Chosen Isolates of *Pedlococcus*

Isolates codes	Mikrobiological indicators CFU/g							
	Total microbe		Group of *coliform*		Histamine producer bacteria		Lactic acid bacteria	
	initiation	end	initiation	end	initiation	end	initiation	end
Pediococcus acidilacticii YDA3	$3.7X10^6$	$8.2X10^9$	$1.6X10^5$	$2.1X10^1$	$2.9X10^5$	$3.2X10^2$	$4.2X10^6$	$8.3X10^9$
Pediococcus pentosus YDA4	$3.6X10^6$	$8.1X10^9$	$1.7X10^5$	$2.6X10^1$	$2.9X10^5$	$3.4X10^2$	$3.8X10^6$	$9.2X10^9$
Control	$3.2X10^5$	$8.1X10^6$	$2.3X10^5$	$7.1X10^2$	$3.3X10^5$	$4.4X10^3$	$2.7X10^4$	$4.1X10^5$

Control: without the inoculation culture of BAL

The process of expense of this proton needs enormous energy, finally the bacteria will die because they worn out of energy (Netcher, 2001). For the peroxide hydrogen that is produced by lactic acid bacteria will produce a very reactive hydroxyl radical and oxidize the cell that causes the damage of structure of protein molecule of bacteria's cell that causes death (Daeschel, *et al.* 1989).

2. The Influence of Starter Cultures towards The Chemical Change

Both of isolates, i.e. *Pediococcus aciddacticii* YDA3 and *Pediococcus pentosus* YDA4 *sp* which were used as the starter cultures of meat fermentation -- *petis* -- caused the chemical change during the fermentation process. The data of the research results of chemical change is presented in the Table 2 as follows:

Table 2. The Chemical Change during The Process of Fermentation with 2 Chosen Isolates of *Pediococcus*

Isolate code	Perubahan kimiawi											
	pH		Total titratable Acid (%)		Dissolved protein (%)		Histamine mg/100g		TVN mg/100g		TMA mg/100g	
	initiation	end	initiation	end	initiation	end	initiation	end	initiation	end	initiation	end
Pediococcus acidifacticii YDA3	6.41	4.52	0.69	0.93	1.61	10.2	2.6	6.2	7.2	42.8	4.2	23.2
Pediococcus pentosus YDA4	6.51	4.47	0.67	0.92	1.62	10.3	2.5	5.8	7.3	43.2	3.9	24.1
Control	6.51	5.78	0.69	0.83	1.68	7.9	2.6	10.6	8.1	87.2	4.3	50.1

Control: without the culture inoculation of BAL

It is seen on the **Table 2** that both of isolates of lactic acid bacteria produce pH (achieves of 4.47- 4.52) compared with the control (only 5.78) and so with a total titratable acid (0.92-0.93%) which is compared with the control (only 0.83%). This shows that both of isolates, i.e. *Pediococcus acidifacticii* YDA3 and *Pediococcus pentosus* YDA4 which are homo-fermentative are able to produce the of lactic acid as controller for undesirable microbes. On the **Table 2,** it is obvious that both of isolates can increase the dissolved protein achieves to 10.2-10.3% which is compared with the control 7.9% only. This is proving that both of isolates have the ability of proteolysis.

Both of isolates also can put pressure on the formed histamine only 5.8 – 6.2 mg/100g compared with the control that achieves 10.6 mg/100g and so, they can put pressure on the formed component indicators of "off-flavor". It is proven by the formed TVN, i.e. only almost half of 42.8 – 43.2 mg/100g compared with a very high of control that achieves of 87.2 mg/100g. So, the formed TMA is smaller only of 23.2 – 24.1mg/100g compared with the control that achieves 50.1mg/100g.

CONCLUSION

The *Pediococcus acidilaotidii* YDA3 and *Pediococcus pentosus* YDA4 can be used as the starter cultures of meat fermentation --*petis* -- because they have added value i.e. they can to minimize on the group of *coliform* and histamine producer bacteria of 1 log cycle; the dissolved protein is increasing up to 10.3%, they can to pressure histamine up to 5.8 mg/100g; they can to pressure TVN up to 42.6 mg/100g; and TMA up to 23.2 mg/100g.

REFERENCES

Ammor, M.S. and Mayo, B. 2007. Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production : an update. Meat Science 76 : 138-146.

AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemist. Association of Official Analytical Chemists, Washington, D.C.

BSN. 1985. Penentuan Kadar TVB dan TVN secara *Conway*. SNI 1-44951985.

Buckenhuskess, L.T. 1993. Selection Criteria for Lactic Acid Bacteria to be Used as Starter Cultures for Various Food Commodities. FEMS. Mic. Rev. 12 : 253-272.

Daeschel, M.A. 1988. Characterization of Bacteriocin from *Lactobacillus plantarum*. Presented at 86-th Annual Mtg. Am.Sc. for Microbiology. Washington. D.C. March (Abstract).

De Vuyst, L.j. 2000. Technology Aspect Related to the Application of Functional Starter Culture. Food Tech & Biotech. 38 : 105-112.

De Vuyst, L.J. 2002. A Novel Area of Predictive Modelling : Describing the Functionality of Beneficial Microorganisms in Foods. Int. J. Food Mic. 73: 251-259.

De Vuyst, L.J. and Leroy, F. 2004. Lactic Acid Bacteria as Functional Starter Cultures for the Food Fermentation Industry. Trends in Food Sc.& Tech. 15:67-78.

De Vuyst, LJj., Verluten, J., and Leroy, F. 2006. Functional Meat Starter Cultures for Improved Sausage Fermentation. Intern. J. of Food Mic. 106: 270-285.

De Vuyst, L.J., Degeest, B., and Leroy, F. 2002. The Functionality of beneficial microorganism in foods. Int. J. of Food Mic. 73 : 253-251.

Egan, A.F. 1983. Lactic Acid Bacteria of Meat and Meat Product. Ant. Van Leuw. 49: 327-336.

Lowry, O.H., Roseborg, N.J., Farr, A.L., dan Randall, R.J. 1951. Protein Measurement with the Folin Phenol Reagent J. Biol. Chem., 193: 265-275

Netcher, E.W. 2001. Microbiology a Human Perspective. 3^{d} ed. Mc. Grave Hill. New York.