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by Hafsan Hafsan

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Potency of Bacteriocin by *Pediococcus acidolacticy* Indigenous Oangke
As Biopreservative for Meatballs

Hafsan¹, Yuyu Ardana¹, Fatmawati Nur¹, Khaerani Kiramang¹ and Muhammad Halifah Mustami²

¹Faculty of Science and Technology Universitas Islam Negeri Alauddin Makassar, Gowa, 92113,

South Sulawesi, Indonesia. Tel: +62-811444881 E-mail: harsan.bio@uin-alauddin.ac.id

²Faculty of Tarbiyah and Teaching Universitas Islam Negeri Alauddin Makassar, Gowa, 92113,

South Sulawesi, Indonesia. Tel: +62-81354634497 E-mail: muhi-halirahmustami@gmail.com

Abstract

This study was aimed to determine the effect of *Pediococcus acidolactici*'s bacteriocins applications originating of dangke to the storage period meatballs. Results has indicated that the applications of bacteriocin have been to inhibit bacterial growth on the meatballs. After the applications of bacteriocin, meatballs stored at room temperature (25°C) for 4 days. Microbiological analysis the addition of preservatives such as bacteriocins and benzoic acid has shown can increase the shelf life or inhibit microbial growth until the fourth day, in other words, can suppress the total number of microbes than without the addition of preservatives (control). Before of storage, the growth of *Escherichia coli* was not found in the meatballs on each treatment. After the storage, qualitatively the meatballs by the addition of acid benzoat nor bacteriocins, *E. coli* was not found until the fourth day, whereas the control meatballs found the presence of *E. coli*. The presence of *Escherichia coli* in meatballs caused by contamination either through the air or during the processing of meatballs. This proves that Bacteriocin by *P. acidolactici* is potential as biopreservative in the product meatballs so that it can be an alternative in the use of food preservatives, however is necessary some research to find culture growth media that is more economical to be applied widely to the public.

Keywords: bacteriocin, *Pediococcus acidolactici*, biopreservative, meatballs.

1. Introduction

The Meatball is one of the processed meat product with a round shape or another, food is highly perishable. To solve this problem, needs a food additive as preservative to prevent the damage caused by microbes, thus remain viable and pose no danger to the health of consumers. Some food additives that often used in making meatballs is nitrite and benzoic acid (chemical legal), borax and formaldehyde (chemical illegally). These materials use to preserve the meatballs that can cause the food to be carcinogenic. Development of food biopreservative (non-chemical preservatives) be an alternative to be used as a preservative agent that will improve the quality and extend the shelf life of food without altering the sensory properties (Fuzaian, 2012). This indicates that the use of biopreservatives is the absolute solution to be encouraged. Biopreservatives should have been put

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into use to substitute harmful preservatives. Safe preservative materials can be derived from microorganisms, such as bacteriocins produced by lactic acid bacteria.

In previous research, been known ability of lactic acid bacteria ie *P. acidolactici* of that have been isolated from Dangke (a distinctive food as a dairy products from Enrekang district, South Sulawesi) produces metabolites in the form of bacteriocins. Therefore, conduct further research to determine the effect of the use of bacteriocins as biopreservatif for food products such as meatballs against microbiological quality, as a measure to determine its potential to be developed as bio preservatif that can be seeded.

Bacteriocins is a protein substance, generally has a low molecular weight and has a bactericidal and bacteriostatic activity. Bacteriocins has been widely used in the fields of antagonistic properties of food preservatives, because of its natural and can be degraded by proteolytic enzymes in the human digestive so as does not harm. The introduction should put the focus of the manuscript into a broader context. As you compose the introduction, think of readers who are not experts in this field. Include a brief review of the key literature. If there are relevant controversies or disagreements in the field, they should be mentioned so that a non-expert reader can find out about these issues further. The introduction should conclude with a brief statement of the overall aim of the experiments.

2. Objectives

This study was aimed to determine the effect of applications *Pediococcus acidolactici*'s bacteriocins that originated of dangke to the storage period of meatballs.

3. Materials and Methods

Material that has been used are *P. acidolactici* pure culture (Indigenous Dangke, collection from Laboratory of Microbiology, Biology Department Faculty of Science and Technology, Alauddin State Islamic University of Makassar), De Man Rogosa and Sharpe Broth media (MRSB), Plate Count Agar (PCA) media, media of eosyn methylene blue agar (EMBA), yeast extract 3%, 1% NaCl, 0.1 N NaOH, ammonium sulfate, potassium fospat buffer and the dialysis membrane. The materials required for making meatballs are beef, tapioca flour, STPP, salt, ice cubes, garlic, benzoic acid and pepper.

o. Production of Crude Bocteriocin Extract by *Pediococcus Acidolactici*

Liter of MRSB be added 3% yeast extract and 1% NaCl, then inoculated with 10% (v/v) culture of *P. acidolactici*, after it was incubated at 37°C for 20 hours (Ogunbawo et al. 2003), then stored at refrigerator of 4°C for 2 hours. Centrifuge at a speed of 10,000 rpm for 20 min at 4°C, and filtering using Sartorius filter membrane, the cell-free supernatant subsequently its pH neutralized to pH 6 using 0.1 N NaOH. 80% ammonium sulfate powder was added gradually to the sterile supernatant antimicrobial. The filtered to produce a precipitate proteins, then homogenized using a stirrer slowly at a 4°C for 2 hours. Furthermore supernatant was discarded and the precipitate obtained bacteriocins. Precipitate was collected on a size 50 ml Falcon tube. Precipitate bacteriocins are dialyzed using a dialysis membrane diameter of 2 cm and buffer used is potassium phosphate for 12 hours, further replacement of twice buffer at 2 and 4 hours at 4°C so that it has obtained extract of bacteriocins crude (Hata et al. 2010).

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b. Making Meatballs

Fresh meat is cut into pieces. The meat was then milled in a food processor by the addition of salt, STTP, $\frac{1}{2}$ part ice cube and added 0.3% bacteriocins. Spices such as pepper, garlic, starch, flavoring and remaining $\frac{1}{2}$ parts of ice be added to the dough. The dough is milled back until evenly mixed and become sticky. The dough is then shaped roundly and boiled at a temperature of 100°C to float (approximately 10-15 minutes). Formulation of meatballs in this study are: fresh meat (62.5%), starch (20% -30%), STPP (0.5% -0.8%), salt (3%-3.5%), ice (35%), garlic (0.75%), pepper (0.75%), and flavorings (0.5%).

c. Total Plate Count Microbes

Amount of 5 g sample meatballs put into a sterile plastic then added 45 ml of 0.85% NaCl sterile, then shaken kneaded to obtain a homogeneous mixture. This sample was then diluted with 0.85% NaCl. Pipette sample aseptically at a dilution of 10^3 to 10^{-5} and 1 ml pipetted into a sterile petri dish and pour the PCA media. Incubation was performed for 24 hours at 37°C. The number of bacteria is determined by the plate count method and formula of determining the number of colonies on each treatment with colony count between 25-250 CFU/g.

d. Quantitative Analysis of *E. coli*

As much 5 grams of sample meatballs put into a sterile plastic then added 45 ml of sterile 0.85% NaCl, then shaken kneaded to obtain a homogeneous mixture. This sample was then diluted with 0.85% NaCl until the fifth dilutions (10^5). Samples were aseptically pipetted in dilution 10^{-1} to 10^{-3} and 1 ml pipetted into pour plate of EMBA media. Incubation was performed for 24 hours at 37°C, *E. coli* colonies growing purplish blue.

4. Results and Discussion

a. Total Plate Count Microbes

Total plate count of microbes need to know to ensure a foodstuffs whether it is feasible or not to be consumed. Microbial growth into foodstuffs are closely related to the amount of water content and storage period.

Table 1 Total plate count of microbes with the addition of Different preservatives on variations of storage period.

Preservatives	Total plate count of microbes $\times 10^5$ (log CFU/g)		
	0	2	4
Control	3.20	4.90	7.25
Acid Benzoate	2.23	5.06	5.15
Bakteriodn	2.65	3.40	4.32

Based on statistical analysis, showed that the preservative and storage time of the total plate count of microbes meatballs was not significantly. But can increase the shelf life or inhibit microbial growth until the fourth day, in other words, can suppress the total number of microbes than without the addition of preservatives. Similarly the addition of benzoic acid as is commonly added in making meatballs.

According to Indonesian National Standard (INS) 01-0366-2000, the maximum limit of the total microbial the product meatballs were 1×10^5 CFU/g. Based on Table. 1, the total number of

microbes on the meatballs with the addition of bacteriocins on days 0, 2, and 4 days still meet SNI, but the meatballs by the addition of benzoic acid on the second day, the total number of microbes meatballs exceeds the limit specified by INS. Total microbial meatballs without preservatives (control) on day 0 is still in accordance with the INS, but on the second day and the fourth, the meatballs have increased the total number of bacteria that exceed the specified limits INS. Total microbes that exceed the normal limits can be caused by the meat used was contaminated with microorganisms and can also be due to contamination of the tools used (National Standard Agency, 2000). In a suitable amount bacteriocins isolated from lactic acid bacteria has the potential to kill and inhibit the growth of pathogenic bacteria (Savadogo et al. 2006).

b. Qualitative Analysis of E. coli in Meatballs

The presence of *E. coli* is one indicator of poor sanitation. *E. coli* is a Gram-negative bacterium, grows optimally at 37°C, but can be grown in the temperature range of 15-45°C (Supardi & Sukanto, 1999). The results showed that the addition of benzoic acid and bacteriocins were able to inhibit the growth of *E. coli* in meatballs compared to controls. This is in accordance with Arief et al. (2008) who stated that the bacteriocins produced by *P. acidolactici* showed antimicrobial activity to inhibit the growth of Gram-negative bacteria, such as bacteriocins is bacteriostatic in Gram-negative bacteria. This is in accordance with Arief et al. (2008) who stated that the bacteriocins produced from *P. acidolactici* showed antimicrobial activity to inhibit the growth of Gram-negative bacteria, such as bacteriocins is bacteriostatic in Gram-negative bacteria. Qualitatively, the meatballs by the addition of nitrite and bacteriocins, *E. coli* was not found until the fourth day, whereas the control meatballs found the presence of *E. coli*. This shows that nitrite and bacteriocins were able to inhibit the growth of *E. coli* during storage.

Usage bacteriocins 0.3% does not cause changes in the physical and sensory characteristics of meatballs. This proves that Bacteriocin is potential as biopreservative in the product meatballs so that it can be an alternative in the use of food preservatives. The mechanism of bacteriocins as biopreservative is by forming a hole in the cell membrane of microbes. Influence the formation of holes cytoplasm is the impact of the bacteriocins that cause changes in membrane potential gradient and the release of intracellular molecules and influx of extracellular substance (environment). The effect causes inhibited cell growth and produces cell death process in which sensitive to bacteriocins. The great advantage the use of bacteriocins especially the origin lactic add bacteria as an antimicrobial substance that is its ability to eliminate microbial pathogens and spoilage of food origin of milk and meat. Possible application is closely related to the characteristics of the antimicrobial substance, which is does not toxic, capable of inhibiting the low levels and is produced by bacteria belonging Generally Recognised as Safe (GRAS) that microbes are does not a health risk.

5. Conclusion

This study has proven that proves that bacteriocin by *P. acidolactici* is potential as biopreservative in the product meatballs so that it can be an alternative in the use of food preservatives, however is necessary some research to find culture growth media that is more economical to be applied widely to the public.

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