ANTIBACTERIAL ACTIVITY OF FERMENTED WHEY BEVERAGE BY PRODUCTS FROM BUFFALO DANGKE

Fatma¹, Ratmawati Malaka¹, Hajrawati¹ and Muhammad Taufik²

¹Faculty of Animal Science, Hasanuddin University Makassar, 90245
²STPP, Gowa
fatma_maruddin@yahoo.co.id

ABSTRACT

Whey derived from by-product processing dangke Buffalo has not been widely utilized in Enrekang. Nutrition components of whey allow to be processed into fermented beverage products. Lactobacillus acidophilus FNCC 0051 is one of the bacteria used for the manufacture of fermented beverage products. The addition of these bacteria can be generated of functional food products. This product can inhibit the growth of pathogenic bacteria (antibacterial). The objectives of the research were to evaluate the antibacterial activity of fermented whey beverage ingredient by-product from Buffalo dangke. The research was conducted according to completely randomized design with a factorial pattern of sucrose level respectively: 0, 9, 12 and 14% as first factors and duration of incubation (4, 8, and 12 hours, respectively) as second factor and 5 replications. Inoculated whey with L. acidophilus then tested with pathogen bacteria (S. aureus FNCC 0047 and E. coli FNCC 0091) to determine the inhibition zone as indicator of antibacterial activity. The results showed that whey fermentation using basic ingredients of buffalo dangke could inhibit the growth of S. aureus or E. coli. Inhibiton zone against pathogenic bacteria significantly enhanced as increasing level of sucrose or incubation time.

Key words: Whey, Buffalo dangke, Fermented beverages, Antibacterial

INTRODUCTION

In Enrekang, buffalo milk has long been used by the public as main ingredient of dangke. Dairy product of dangke was similar with soft cheese produced without fermentation process and serve as traditional and typical local food. Dangke processing byproduct is whey dangke. Whey is generally thrown away.

Whey is derived from processing of buffalo dangke byproducts. Nutrition values of whey were potentially to processed be valuable products i.e. beverage products which are a high value and demand in today’s society (Gallardo - Escamila et al., 2007). The product preferences were based on the amount of lactose in the whey (4.6-5.2%) (Panesar et al., 2007; Fatma, et al., 2012ab).

Lactose can be used by common microorganism that used in the manufacture of fermented beverages e.g. Lactobacillus acidophilus (de Castro, et al, 2009; Pescuma et al., 2010). L. acidophilus was classified as probiotic bacteria that provide a variety of benefits to human health (Gambelli, et al., 1999; Lucas, et al., 2004; Almeida, et al., 2008).
Processing of whey derived from buffalo dangke into functional food products is also based on the shift of the current food philosophy to improve of consumers' health. The aim of the research was to evaluate antibacterial activity of fermented whey beverage as by-product of production of Buffalo dangke.

MATERIALS AND METHODS

Experimental designs. The research was conducted according to completely randomized design with factorial design (4 x 3) with 5 replications. The first factor was levels of sucrose (0, 9, 12, and 14% respectively), and second factor was duration of incubation (4, 8, and 12 h, respectively). Post hoc test of Least Significant Difference was used to differentiate the different effect among the treatments (Sudjana, 2002).

Manufacture fermented beverages. Whey obtained from manufacture of buffalo dangke in Curio District of Enrekang Regency. Whey mixed with tapioca flour 0.7% (w/v) until well-mixed and measured as initial volume. Whey mixture then heated and added sucrose (0, 9, 12, and 14% respectively) while stirring for 5 minutes at a constant temperature (70°C). Following heating, distilled water was added until the volume reaches initial volume before heating. Whey subsequently was pasteurized at a temperature of 80°C for 30 min (modified from Alakali et al., 2008), inoculated with starter bacteria (Lactobacillus acidophilus FNCC 0051) and incubated at 37°C for 4, 8, and 12 h, respectively.

Antibacterial activity measurement. Inhibition zone test on solid medium (well diffusion method) used for the measure of antibacterial activity of fermented beverages against two bacteria species: Staphylococcus aureus FNCC 0047 and Escherichia coli FNCC 0091. Sterilized ring wells (9.6 mm diameter inserted into the petri dish). Liquid nutrient agar (40-45°C) with pathogenic bacteria cultures inoculums were uniformly spread in petri dish agar with sterile ring and allowed to solidify. Well ring was then removed using sterile tweezers. 550 μL of fermented beverages samples were inserted into the ring hole and further incubated for 24-48 hours at 37°C under aerobic condition. Diameter of the clear zone formed was measured by caliper. Area of the whole zone was calculated and then reduced pitting area and the results were reported as the zone of inhibition (in mm) (modified form Seydim and Sarikus, 2006).

RESULTS AND DISCUSSIONS

Antibacterial activities of fermented whey against gram-positive bacteria

Antibacterial activity of fermented whey against pathogenic test-bacteria was seen with the magnitude of inhibition zone diameter (Tables 1 and 2). The research showed that fermentation of whey using L. acidophilus FNCC 0051 significantly inhibited the growth of S. aureus and E. coli. This phenomenon is in line with studies using other fermentation product such as L. acidophilus strain LB (Coconnier et al., 1997), or L. acidophilus n.v Er 317/40 strain Narine (Mkrtchyan et al., 2010). Antimicrobial activity of these product exhibited broad spectrum activity against tested isolates of bacteria, such as S. aureus, L. monocytogenes, S. typhimurium, Shigella.
Inhibition zone (mm) whey fermented with various levels of sucrose and different time of incubation against S. aureus (Gram positive).

<table>
<thead>
<tr>
<th>Level of sucrose (%)</th>
<th>Time of incubation (hours)</th>
<th>Mean</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>7.90a</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>9.31b</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>9.39b</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>13.07e</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>9.67a</td>
</tr>
</tbody>
</table>

The inhibition zone significantly increased as the increasing level of sucrose or the length time of incubation (Table 1). Further analyses showed that effect of sucrose level on inhibition zone against S. aureus increased in line with the increasing of incubation time. This phenomenon suggests that sucrose may act as an antibacterial against S. aureus. Antibacterial activity of fermented whey beverage products was also alleged by the existing bioactive components in whey e.g. bacteriocins and lactic acid.

Antibacterial activities of fermented whey against gram-negative bacteria

Possessed antibacterial activity of fermented whey against tested E. coli was indicated by the magnitude of inhibition zone diameter. Inhibition zone increased along with increased levels of sucrose in whey fermentation (Table 2).

<table>
<thead>
<tr>
<th>Level of sucrose (%)</th>
<th>Time of incubation (hours)</th>
<th>Mean</th>
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<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>8.04a</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>15.22c</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>16.71c</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>19.31b</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>9.29a</td>
</tr>
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</table>

Sucrose levels, incubation time and their interaction, significantly increased inhibition zone of fermented whey against E. coli (Table 2). The adverse effect of sucrose levels on E. coli growth was enhanced by the length of incubation time. These results were far greater than the results reported by Fatma (2012). This is allegedly due to differences in bacterial species, differences in the concentration of whey fermentation that inserted into the wells and the difference components of the building blocks of whey fermentation (sucrose level). Supardi and Sukamto (1999) suggested that the use of high concentrations of sucrose (at least 40% dissolved solids) in food processing, caused some of the water in the material becomes available for the growth.
of microorganisms, and water activity ($a_w$) of the material decline causing microorganisms were not capable of performing their activities.

Sucrose is one of the preservative agents in food processing by inhibiting the activity or the growth of bacteria. The addition of sucrose led to hypertonic environment, the cell of bacteria has a lower concentration of solutes than the surrounding extracellular fluid, and water diffuses out of the cell by osmosis. Moreover, cells of bacteria become dehydrated and causing the death. Increased level of sucrose during incubation, activity against bacteria enhanced due to higher rate of osmosis.

In addition, antibacterial activities of fermented whey beverage products were derived from lactic acid as the primary metabolites. Lactic acid is the end product of carbohydrate metabolism by L. acidophilus in the whey beverage products (Pescuma et al., 2010). Rahman et al. (1992) suggested most of microorganisms grow well in neutral pH (6.6 to 7.5). Lactic acid concentrations causing pH decline and retard the growth of microorganisms. Supardi and Sukamto (1999) argued that the acidification of food for at least has two antimicrobial properties i.e. the effect on pH value and the nature of poisoning activities of this acid.

Bacteriocin as secondary metabolites from L. acidophilus was also reported responsible for antibacterial activity of beverages products (Karthikeyan and Santhosh, 2009; Ahmed et al., 2010). Pawiroharsono (2007) suggested that secondary metabolites are compounds that are synthesized by microbes but not for physiological needs. Jack et al. (1995), Knoll et al. (2007), and Ahmed et al. (2010) stated that most of gram-positive bacteria produce bacteriocins that contain 30 to 60 amino acids with activities varying from narrow to broad spectrum against bacteria. Inhibitory effects of L. acidophilus bacteriocins that reported by Karthikeyan and Santhosh (2009), showed that these bacteriocins effectively inhibit 10 species of subsequent bacteria: B. subtilis, S. aureus, L. bulgaricus, S. typhimurium, S. paratyphi B, E. coli, Klebsiella sp, Serratia marcescens, Pseudomonas aeruginosa and Vibrio cholerae.

Other minor bioactive components contained in whey such as lysozyme, lactoperoxidase and lactoferrin were also reported to have antimicrobial activity to inhibit the growth of pathogenic bacteria. Fardiaz et al. (1992), Madureira et al. (2007), and Naim (2008) suggested that lactoferrin is an iron-binding glycoprotein. Most microorganisms require iron for growth. Lactoferrin has the potential to inhibit (bacteriostatic) or to kill (bakteriolisis) bacteria by interfere the iron needs of the bacteria. Lysozyme kills bacteria by disruption of glycosidic bond formation between the two components of peptidoglycan found in bacterial cell walls. Lactoperoxidase has the potential to inhibit or even to kill bacteria by oxidizing sulfhydryl groups of the cell membrane. Lactoperoxidase generally behave as bacteriostatic against Gram-positive bacteria or as bakteriolisis against Gram negative bacteria.

**CONCLUSION**

Increased levels of sucrose and the incubation time led to greater antibacterial activity of whey fermentation against both gram-positive bacteria (S. aureus) and gram-negative bacteria (E. coli).
REFERENCES


