

THE OCCURRENCE OF YEASTS AND FUNCTIONAL PROPERTIES OF INDONESIAN ETHNIC FERMENTED FOODS AND BEVERAGES

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Abstract

Indonesia has a great diversity of ethnic fermented foods and beverages. Besides tempe, there are many famous fermented ethnic products in Indonesia, some of them are Tape', Dadih, Dangke, Bakasam and Sie Reuboh. Tape' is ethnic fermented cassava which fermented by the consortium of lactic acid bacteria, yeast and mould contained in ragi. Dadih is buffalo milk that naturally fermented inside the bamboo, while Dangke is buffalo milk which curdled using papaya latex and then fermented in coconut shell. Bakasam is meat which anaerobic-naturally fermented with the addition of rice, salt and sugar. Meanwhile, Sie Reuboh is meat that fermented with the addition of palm vinegar.

Even yeasts were not the main microorganisms in the fermentation process, their functional properties were highly recognized in resulting the important foods characteristics. The yeasts isolated from Tape' such as *Saccharomycopsis fibuligera* shown great potential of antimicrobial and proteolytic activities (Roostita, et al., 2011). *Candida curiosa*, *Brettanomyces custersii* and *Kluyveromyces lactis* isolated from Dadih shown an antimicrobial activities towards *B. subtilis*, *E. coli* and *S. aureus* (Yurliasni, 2010). Dangke also shown antimicrobial activities towards *E. coli* and *S. aureus*. The yeasts population isolated from Bakasam and Sie Reuboh shown proteolytic activities.

The functional properties of yeasts that isolated from Indonesian ethnic fermented foods were originally has great potential to develop as commercial products. Bioactive compound that showing antimicrobial activities towards the pathogenic bacteria can be purified and developed as food bioperservatives or even nutraceutical products. Meanwhile the proteases produced by yeasts also could developed as local enzyme that could reduced the dependency to the imported enzyme.

Key words: Indonesia, Ethnic Fermented Foods, Beverages, Functional properties.

INTRODUCTION

Indonesia is a great country in South East Asia which have a great diversity of ethnic, culture and also ethnic foods and beverages. More than 200 millions population and 1.100 ethnic in Indonesia which possible to have more than 5.500 ethnic foods and beverages. Some of the foods and beverages produced by using fermentation process which resulting well-known and liked products because of their unique characteristics.

Microorganisms has important roles in specify the characteristics of ethnic foods and beverages produced. Ethnic foods and beverages usually made by natural spontaneous fermentation that involving mixed-culture with the main role of one strain of the microorganism and the other strain role as contaminants (Pawiroharsono, 2007). The

contaminants in ethnic food and beverages fermentations does not always give harmful effects, many of them generate good effects for the products (Yuan, 1999).

There are many famous Indonesian ethnic fermented foods and beverages. One food that well-known worldwide was tempe which resulted from soybean fermentation using *Rhizopus sp.* especially *R. oligosporus*, *R. oryzae*, *R. Arhizus*, *R. Stolonifer* and *R. microsporus* (Astuti, et al., 2000). Meanwhile domestically, other fermented foods and beverages such as Tape', Dadih, Dangke, Bakasam and Sie Reuboh were also liked by the peoples and the number of consumption was relatively high.

All of Indonesian ethnic fermented products mentioned above, showing the presence of microorganisms diversity that involve in the fermentation process. Tape' was cassava which

fermented with addition of dried mixed starter which called *ragi tape* that naturally contains filamentous fungi, yeast and bacteria (Sujaya, et al., 2002; Sujaya, et al., 2010). Meanwhile domination of lactic acid bacteria were found in *Dadih*, *Dangke*, *Sie Reuboh* and *Bakasam* with the important role of yeasts which resulting specific characteristics of the products (Surono, 2003; Suhairi, 2007; Yurliasni, 2010; Wikandari, et al., 2012; Kesuma, et al., 2013). The presence of yeasts in ethnic fermented foods and beverages are mostly role as contaminants that have been widely studied as giver of flavors and accelerate the maturity of the products (Wyder & Puhan, 1999; Roostita & Fleet, 1996). The population mostly range from 10^6 - 10^7 cfu/g and has an important activity in acid metabolism so as to raise the pH and has biochemical activity that produces effects on the food products (Fleet, 1990; Heard & Fleet, 1999).

Besides give good effects towards products characteristics, the presence of yeasts in ethnic fermented foods and beverages also shown functional effects. *Saccharomycopsis fibuligera* from *Tape* shown antimicrobial and proteolytic activities (Roostita, et al., 2011). Indigenous *Dadih* yeasts such *Candida curiosa*, *Brettanomyces custersii* and *Kluyveromyces lactis* shown an antimicrobial activities towards *B. subtilis*, *E. coli* and *S. aureus* (Yurliasni, 2010). *Dangke* as a products could decrease the activities towards *E. coli* and *S. aureus* and 0.06 - 2.89×10^4 cfu/g yeasts population isolated from *Bakasam* and *Sie Reuboh* shown protelytic activities (Roostita, et al., 2009).

The functional properties of indigenous yeasts and Indonesian ethnic fermented foods were originally has great potential to develop as commercial products. Bioactive compound that showing antimicrobial activities towards the pathogenic bacteria can be purified and developed as food bioperservatives or even nutraceutical products. Meanwhile the proteases produced by yeasts also could developed as local enzyme that could reduced the dependency to the imported enzyme.

Yeasts Occurrence and Functional Properties in Indonesian Ethnic Fermented Cassava

Many kind of Indonesian ethnic fermented foods and *Tape* is one of the most popular among them. *Tape* made from cassava that fermented with dried mixed starter which called *ragi tape* that naturally contains filamentous fungi, yeast and bacteria (Sujaya, et al., 2002). Microorganisms especially yeasts that live on *Tape* utilize simple and complex sugars as their carbon source (Lewis & Young, 1990).

Tape is potential as yeasts habitat. Yeasts population of 2×10^6 cfu/g was found and shown antimicrobial activities in *Tape* (Roostita, et al., 2011). Beside that, yeasts also generate proteolytic activity by producing extracellular protease (Roostita & Fleet, 1996). *Saccharomycopsis fibuligera* strain R64 were one of isolated yeast from *tape* that produced extracellular protease with optimum pH of 5 and temperature of 25°C (Roostita, et al., 2012). The extracellular protease produced by yeasts is well known and many people utilized it for their activities. Yeasts extracellular protease has potential in beer and wine stabilization (Ogrydziak, 1993). Proteolytic enzymes have some important role in medicine such as food digestion, protein turnover, blood coagulation, embryonic development and cell division (Reid, 2012). Therefore, the enzymes were an important group in scientific, medical research and biotechnology (Rawlings, et al., 2009).

The Role of Yeasts and Functional Properties of Indonesian Ethnic Fermented Milk

Dadih, an Indonesian ethnic fermented milk of West Sumatra is made by pouring fresh raw unheated buffalo milk into a bamboo tube capped with banana leaves, and allow to ferment at room temperature for two days. The use of buffalo milk in West Sumatra aims to exploit abundant buffalo milk. Buffalo milk are less preferred when consumed in a fresh state because of the smell. Fermentation is done so the flavour will be more acceptable.

The making of *Dadih*, involves several kinds of microorganisms including lactic acid bacteria (LAB), molds and yeasts. The existence of yeasts in the fermentation of *Dadih* should be considered, because it can make a positive contribution during the fermentation process and end products such provide growth factors for other microorganisms and also as flavor enhancer. In addition secondary metabolites produced by yeasts such as acetate, succinate, propionate, fumarate and piruvat has a good influence on the taste and have the ability as antimicrobial which can inhibit the growth of pathogenic bacteria.

There are three potential yeasts isolated from *Dadih*, such as *Kluyveromyces lactis*, *Candida curiosa*, and *Brettanomyces custersii*. *Kluyveromyces lactis* has strong antimicrobial activity against *B. subtilis* with clear zones of inhibition 5mm, *C. curiosa* against *E. coli* with inhibition zone 5mm, and *C. curiosa* and *Brett. custersii* against *S. aureus* with clear zones of inhibition respectively 5.75mm and 7mm which showed that the yeast isolated from *Dadih* is able to inhibit the growth of pathogenic bacteria (Yurliasni, 2010).

C. curiosa, *Brett. custersii* and *Kluy. lactis* have strong activity against gram-negative bacteria compared to gram-positive bacteria. Antimicrobial activity would be seen when the interaction between yeast and bacteria occur (Golubev & Boekhout, 1992). The interaction not only indicate a positive or negative traits of fermentation process, but involves antagonistic activity against yeasts and other microorganisms with produce micocin (anti-microbial compounds).

Different with *Dadih*, *Dangke* is an Indonesian ethnic fresh soft cheese that is usually made from fresh cow milk or buffalo milk by the farmers' households in Enrekang regency, South Sulawesi province. *Dangke* made by heating with a small fire to boil, then add coagulant in the form of sap of papaya (papain) resulting in natural clotting which change the cow's or buffalo milk become solid due to the separation of protein and water (Rahman, 2013).

Cow milk *Dangke* has a high nutrient content (water content of 55%, protein of 23.8%, fat of

14.8% and ash of 2.1%) and its near normal pH value of 6.4 (Hatta, et al., 2013). Its shelf life is generally two days at room temperature, while at the refrigerator temperature, it can reach five to seven days. Preservation method is usually done by the community is the addition of salt solution.

Functional properties of *Dangke* shown by the microorganisms involves in the fermentation process. Some of microorganisms such lactic acid bacteria isolated from *Dangke*, producing bacteriocin that could inhibit pathogenic bacteria *Salmonella typhiimurium* (Razak, et al., 2009). As well as yeasts capable of producing metabolites and create condition that is not conducive for harmful microorganisms such as *E. coli* and *Salmonella spp.* which is the main contaminant of *Dangke* (Hatta, et al., 2013)

Functional Properties and Yeasts Role in Indonesian Ethnic Fermented Meat

Bakasam is ethnic fermented meat from Lampung, Indonesia. It has acid flavor. The ingredients of *Bakasam* were 20 gram of rice, 2 gram of salt, 0.2 gram of sugar, and 100 gram of top side meat. The fermentation carried out until 15 days under anaerobic condition. The microorganism grew in anaerobic situation of fermentation process were the bacteria, yeasts, or mold (Buckle, et al., 1987; Winarno & Fardiaz, 1993).

Yeasts was able to grow in many products including fresh meat and its processed products (Roostita, 2004). Meat was beneficial since it contained the nutrient needed by the body. Some yeasts found in fresh meat were *Candida*, *Debaryomyces*, *Rhodotorula*, and *Torulopsis*; while large number of yeasts found in processed meat and cured yeast meat were *Candida*, *Torula*, *Torulopsis*, *Trichosporon* dan *Debaryomyces* (Dwidjoseputro, 2003; Jay, 1996; Roostita, 2004). Fermentation process appeared as a result of anaerobic type of metabolism.

The growth of yeasts in *Bakasam* increased until day 3 (23.91×10^4 cfu/g), and then it decreased until day 15 (0.46×10^4 cfu/g). The amount of total yeasts with proteolys activity found in *Bakasam* meat ranged from

0.06×10^4 cfu/g to 2.89×10^4 cfu/g. The growth of yeasts increased until day 6 (2.89×10^4 cfu/g), and then it decreased until day 15 (0.21×10^4 cfu/g) (Roostita, et al., 2009).

At the early stage of fermentation, the number of total yeast with proteolysis activities was found to be the least. It was because the yeasts with proteolysis activities just recently broke the protein inside the meat especially proteolysis one, used protein as their energy source (Soeparno, 2005).

It happened since the yeast with proteolysis activities experienced their growth process by breaking the protein inside the meat. The activity was assumed as the activity of protease enzyme that came from high number of yeast. Then, these enzymes experienced autolysis that resulted in high number of yeasts death. Thus, high numbers of yeast colonies were found. Yeast with intracellular proteases activity was contributed in the presence of proteolysis activity (Roostita, 1993). The yeast produced the protease enzyme not only outside the cell, but also inside the cell (intracellular activity) that can only be seen and be measured only if the autolysis from its yeast cell occurred (Roostita, 2004).

Different with *Bakasam*, *Sie Reuboh* is a beef or buffalo meat products from Aceh which manufactured by using ingredients such as vinegar, fat, salt and spice inside the slice meat and then heated (Suhairi, 2007). Beside the proteolytic yeasts that grew in the beef or buffalo meat, acetic acid bacteria in vinegar were contribute in making acid condition that is not suitable for spoilage and pathogenic microorganisms. *Saccharomyces spp.* and *Acetobacter spp.* dominantly grew in vinegar that added into beef or buffalo meat which will be made *Sie Reuboh* resulting in functional effects in the end products.

CONCLUSIONS

Indonesian ethnic fermented foods and beverages such *Tape'*, *Dadih*, *Dangke*, *Bakasam* and *Sie Reuboh* shown presence and role of yeasts which determined from the products characteristics. Yeasts presence gave great potential of functional effects to

developed i.e. proteolytic activities and antimicrobial activities towards spoilage and pathogenic bacteria. The metabolites (extracellular proteolytic enzymes, antimicrobial compound) produced by yeasts still need to be developed so that could give best functional effects if it is produced as commercial products.

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REFERENCES

- Astuti M., Meliala A., Dalais F.S., Wahlqvist M.L., 2000. Tempe, a nutritious and healthy food from Indonesia. Asia Pacific Journal of Clinical Nutrition, Volume 4, 322-325.
- Buckle K.A., Edward R.A., Fleet G.H, Woutton I., 1987. Food Science. Second ed. Jakarta: Universitas Indonesia.
- Dwidjoseputro D., 2003. Dasar-dasar Mikrobiologi. Jakarta: Penerbit Djambaran.
- Fleet G.H., 1990. Yeasts in Dairy Products. Journal of Applied Biotechnology, Volume 68, pp. 199-211.
- Golubev W., Boekhout T., 1992. Dimorphism in *Intersonilia perplexans*: yeasts and hyphal phases differ in their sensitivity to mycocins produced by tremellaceous yeasts. FEMS Micriobiol. Let., Volume 98, 187-190.
- Hatta W., Sudarwanto M., Sudirman I., Malaka R., 2013. Prevalence and Sources of Contamination of *Eschericia coli* and *Salmonella spp.* in Cow Milk Dangke, Indonesian Fresh Soft Cheese. Global Veterinara, 11(3):352-356.
- Heard G.M., Fleet G.H., 1999. Yarrowia (*Candida*) *lypolitica*, Sydney-Australia: Department of Food Science and Technology, The University of New South Wales.
- Jay J.M., 1996. Modern Food Microbiology. Fifth Edition ed. New York: Chapman and Hall.
- Kesuma F.M.V., Sayuti S.M., Al-Baarri A.N., Legowo A.M., 2013. The characteristic of Dangke made from milk with different incubation time after dipping with lactoferrin. Journal Aplikasi Teknologi Pangan, 2(3):155-158.
- Lewis M.J., Young T.W., 1990. Brewing. New York: Chapman and Hall.

- Ogrydziak D.M., 1993. Yeasts extracellular proteases. *Biotchnology*, Vol. 13, 1-55.
- Pawiroharsono S., 2007. The development of industry and bioeconomy based on traditional fermented foods. *Jurnal Ilmu Kefarmasian Indonesia*, 5(2):85-91.
- Rahman S., 2013. Development Dangke as Potential Local Milk Products from Enrekang District. *Jurnal Aplikasi dan Teknologi Pangan*, 3(1):41-44.
- Rawlings N.D., Barret A.J., Bateman A., 2009. *MEROPS : The Peptidase Database*, Cambridge: Babraham Institute.
- Razak A.R. et al., 2009. Production of Bacteriocin by Fermentation using LAB *Enterococcus faecius* DU55 isolated from Dangke. *Jurnal Akta Kimia Indonesia*, 2(2):1-9.
- Reid V.J., 2012. Extracellular acid proteases of wine microorganisms: Gene identification, activity characterization and impact on wine, Matieland - South Africa: Stellenbosch University. Institute for Wine Biotechnology, Faculty of AgriScience.
- Roostita, 1993. Occurrence, growth and biochemical properties of yeasts in cheese and milk, New South Wales: The University of New South Wales.
- Roostita, 2004. Potensi dan prospek yeasts (khamir) dalam meningkatkan diversifikasi pangan di Indonesia, Bandung: Universitas Padjadjaran.
- Roostita L.B., Putranto W.S., Lelono D., Andriansyah P., 2009. Stud of biochemical properties of lactic acid bacteria and yeasts in Bakasam Indonesian Fermented MEat. Tehran - Iran, International COngress of Food Hygiene.
- Roostita R., Fleet G.H., 1996. The Occurrence and growth of yeasts in Camembert and Blue veined cheese. *International Journal of Food Microbiology*, Vol. 28, 393-404.
- Roostita T.B. et al., 2011. Isolation and characterization the potential yeasts isolate for antimicrobial compound production from Indonesian fermented foods. Guadalajara-Mexico, 29th International Specialized Symposium on Yeasts.
- Roostita T. et al., 2012. Characterization of extracellular protease from *Saccharomycopsis fibulgera* strain R64. Istanbul, FoodMicro 2012.
- Soeparno, 2005. *Ilmu dan Teknologi Daging*. Fourth Edition ed. Yogyakarta: Gadjah Mada University Press.
- Suhairi L., 2007. The effect of repeated on nutrient content and acceptance of Aceh traditional food (Sie Reuboh), Bogor: Thesis - Institut Pertanian Bogor.
- Sujaya I.N. et al., 2002. Specific enumeration of lactic acid bacteria in ragi tape by colony hybridization with specific oligonucleotide probes. *World J. Microbiol. Biotechnol.*, Vol. 18, 263-270.
- Sujaya I.N., Nocianitri K.A., Asano K., 2010. Diversity of bacterial flora of Indonesian ragi tape and their dynamics during the tape fermentations as determined by PCR-DGGE. *International Food Research Journal*, Vol. 17, 239-245.
- Suroño I.S., 2003. In vitro probiotic properties of indigenous Dadih lactic acid bacteria. *Asian-Australasian Journal of Animal Sciences*, 16(5):726-731.
- Wikandari P.R., Suparno Marsono Y., Rahayu E.S., 2012. Characterization of proteolytic Lactic Acid Bacteria fro Bekasam. *Jurnal Natur Indonesia*, 14(2):120-125.
- Winarno F.G., Fardiaz, 1993. *Biofermentasi dan Biosintesa Protein*. Bandung: Penerbit Angkasa.
- Wyder T.M., Puhan Z., 1999. Role of selected yeasts in cheese ripening : an evaluation in aseptic cheese curd slurries. *International Dairy Journal*, Vol. 9, 117-124.
- Yuan R., 1999. The fermentation of soy sauce : a traditional approach or high-tech process. *The Diversity Notebook*, September.
- Yurliasni, 2010. Antimicrobial activity of yeast dadih (buffalo milk fermentation) origin on pathogenic bacteria. *Agripet*, 10(1):19-24.