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Study of the Quality of Multi Probiotic Fermented Milk Made from Cow's Milk and Goat's Milk

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ABSTRACT: This objective of this study was to evaluate the growth of multi probiotic bacteria on fermented cow and goat milk including pH, acidity, and some biochemical changes on organic acid and sensory properties. These probiotic bacteria used were *Lactobacillus acidophilus, bifidobacterium longum*, and l*actobacillus casei* mixed (abc culture) at 10% v/v. Analysis of pH's value and acidity were conducted at 0, 3, 6 and 9 hours of incubation using pH meter and titration while organic acid were conducted using HPLC at early and end of incubation. The results showed that the acidity and pH of the product were (0.59; 0.60) and (3.40; 4.30), either cow's milk or goat's milk indicated the growth of mixed multi probiotic bacteria. It has also presented that different milks have significantly effects ($P<0.05$) on values of pH, lactic acid content as well as the quantity of sensory. This study have presented the opportunity to develop new product using multi probiotic bacteria in cow's and goat's milk that is considered satisfactory and accepted by consumers.

Keywords: probiotic bacteria, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium longum*, fermented milk

INTRODUCTION

The main role of diet is to provide nutrients to meet host physiological requirements. As research behind diet and health has evolved, so has the concept of 'functional foods' become more and more popular. Foods which are considered as being 'functional' are thought to exert certain positive properties over and above their normal nutritional value. The concept has now moved markedly towards gastrointestinal function, in particular the impact of gut bacteria. The gut microflora contains pathogenic, benign and beneficial microbial species. Functional foods directed towards the gut microbiota would serve to influence the composition of activities towards a more positive metabolism (Gibson, 2007).

In recent years, increased knowledge and understanding of gut micro-flora composition and activities has made the concept of functional food move markedly towards gastro-intestinal function which beneficially affect gastrointestinal function by influencing its compositions (Gibson, 2007). It sold mainly as ingredients in fermented food and almost exclusively consumed as fermented dairy products such as fermented milk (Soccol *et al*., 2013).

Dairy products containing probiotic cultures such as bifidobacteria, *Lactobacillus acidophilus*, and *Lactobacillus casei*-selected because of their health-promoting properties-and have been produced for many years. The health and nutritional benefits ascribed to these probiotic bacteria include the alleviation of lactose intolerance, inhibition of pathogenic microorganism and viruses, and prevention of diarrhea. The use of milk (e.g. cow's and goat's milk), in combination with bacterial strains having probiotic properties and/or producing physiologically active metabolites, represents one of the technology options for manufacturing new dairy functional beverages (Vinderola *et al*, 2000).

The development of dairy products containing probiotic bacteria (*Bifidobacteria* and internal *Lactobacilli*) is, currently, an important topic with industrial and commercial consequences. Most of the work of probiotic fermented milk has been carried out with bifidobacteria alone, or mixed cultures of *Bifidobacteria* and *L. acidophilus*, but *L. casei* has rarely been used. This objective of this study was to evaluate the growth of multi probiotic bacteria (*Bifidobacteria*, *L. acidophilus*, and *L. casei*) on fermented cow and goat milk including pH, acidity, organic acid, and sensory properties.

MATERIALS AND METHODS

Strain of probiotic bacteria: *Lactobacillus acidophilus* FNCC 0051, *Lactobacillus casei* FNCC 0090 and *Bifidobacterium longum* ATCC 15707 belonging to the culture collection of Pusat Studi Pangan dan Gizi (PSPG) Laboratorium Pangan dan Gizi Universitas Gadjah Mada. Fermented cow and goat milk culture was prepared by heating raw milk at 110°C for 15 minutes. Single and mix cultures developed in Dairy Science and Milk Industry Laboratory (ISO 17025:2005) Universitas Gadjah Mada. The culture cultivated as 10% v/v and incubated at 39°C for 9 hours.

The pH and acidity were detected each 3 hrs of incubation. The pH was measured with a pH meter while acidity was measured in 9 ml of culture after adding 0.5 ml of a 1% solution of phenolphthalein in 95% alcohol, by titrating with 0.1 N NaOH. Organic acid was detected by using HLPC LC column Shin-pack VP-ODS (Shimadzu) at 254 nm wave length, 30o C, flow rate 0.4 ml/minute. The sensory evaluation test had been conducted by twenty entrained panelists using five scale of intensity or score (Murti *et a*l, 1993).

Statistic. Data from growth of bacteria and organic acid were analyzed by Factorial design procedure of SPSS software. The differences among means were detected by Duncan's multiple range test.

RESULTS AND DISCUSSION

Growth of bacteria

The performance of the growth probiotic bacteria was (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium longum*) was assessed as the ability of strain to produce organic acids as the primary metabolites, which was measured by pH decline and acidity in various incubation time. The change of pH and titrable acidity during incubation is presented in figure 1. During 9 hours of incubation of mixed culture bacteria in sterile cow's and goat's milk, these culture induced pH reduction in various degrees ranging from 6.0 to 3.4.

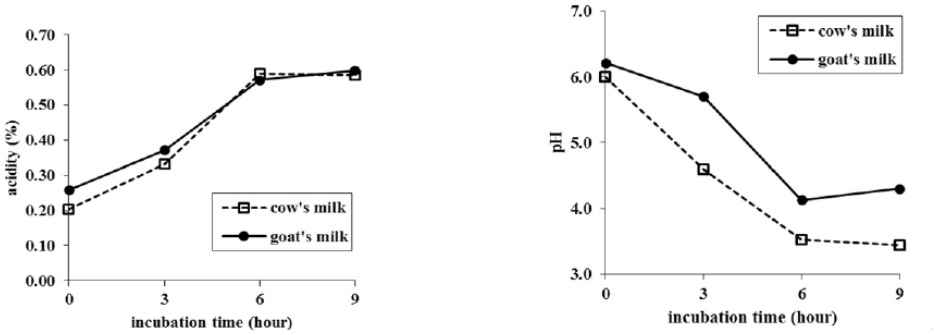

Figure 1. pH value and titrable acidity during 0, 3, 6 and 9 hours incubation

Growth of mixed culture bacteria can also be represented by an increase of acidity. Moreover, measuring acidity considerable advantage for monitoring or controlling acidification processes. The final acidity value of Fermented cow's milk and goat's milk increase to 0.6 % (fig 1). Acidity values during incubation increase as a result of bacterial growth that produce lactic acid or acetic acid depended on type of LAB used (Habibah and Kadhafi, 2011).

The result indicated that mix culture of abc could growth both in goat and cow milk. Growth mix culture abc in cow milk was relatively better than in goat milk. The pH decline depends on the amount of lactic acid and other organic acid released, which is directly linked to the culture metabolic activity. Somr of them produce mainly lactic acid (*L. acidophilus* and *L. casei*) while other *B. longum* produce also acetic acid. Certain lactic acid bacteria strains can utilize lactose fully as opposed to some others than can mainly convert a part of lactose into lactic acid whitch could have been reason for a slow pH decline (Donkor, 2007).

Organic acid

Changes in lactic acid content as determined by HLPC are shown in fig. 2. The amount of lactic acid produced at the end of fermentation depended on the type of milk used, and it ranged from 303.56 (cow's milk) to 1018.70 mg/L (goat's milk). Lactic acid content in fermented goat's milk the increased during fermentation from 913.62 to 1018.70 mg/L. The homofermentative bacteria *L. acidophilus* and *L. casei* in pure or in co-culture produced the largest amount of lactic acid bacteria using the Embden-Meyerhof-Parnas pathway (glycolysis), while *Bifidobacterium longum* is an heterofermentative strain that ferments lactose through a specific route called bifidus pathway. Theorically, the fermnentation of two glucose molecules leads to 3 mols of acetic acid and 2 mols of lactic acid (Cassarotti *et al*, 2014).

The change of acetic acid that earlier than lactic acid indicated that bacteria producing acetic acid have growth quickly and early than other bacteria. As we know bifidobacteria is the only one bacteria in mix culture that produce acetic acid.

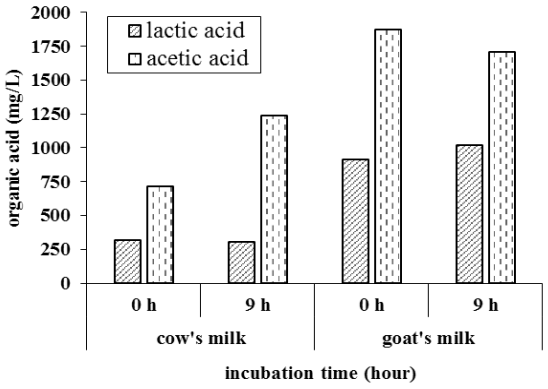

Figure 2. Consentration of organic acid during 0 and 9 hours incubation

Sensory evaluation

The acceptability of consumers can be seen as in figure 3 in which severally were disliked by consumers. It is showed that goat's milk sample produced high bitter taste and cow's milk sample had higher acid taste. Despite of sensory evaluation, it showed that fermented cow milk mix culture of three probiotic bacteria has been accepted better than fermented goat milk.

As the acidity raised milk probably aggregated or coagulated. It is noted that when the acidity reached 4.5-4.6 fermentation of milk would cease and the mineral would release as well as the hydrophobic amino acid giving a possible bitter and salty taste of end products due to the released of the two components (Murti, 1995).

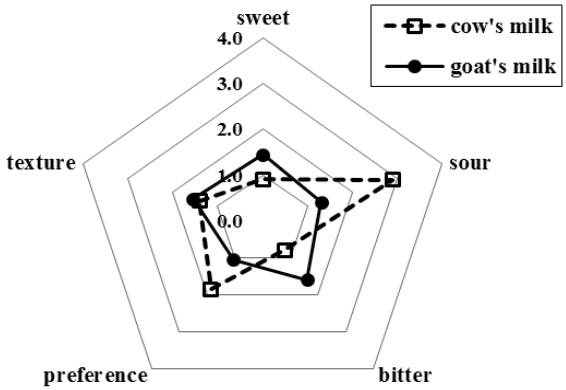

Figure 3. The sensory evaluation of fermented milk samples

L. acidophilus, *L. casei* and *Bifidobacterium longum* which used as the starter to make fermented milk gave significant effect to consumer acceptance. Acid flavour shown significantly more than other flavour and increased consumer likes.

CONCLUSION

It concluded that mix culture of three probiotic bacteria (abc) have grown in cow and goat milk, lead to change organic acids that produced and sensory acceptance by entrained panelist. It also suggested to the next research making more clearby biochemical reaction especially cooperation among bacteria, either commensalism or proto cooperation.

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