Abstract
Dadih is a traditional fermented food of West Sumatra, Indonesia that is considered being able to provide health benefits. Fermentation starter used a combination of *L. casei* and *L. plantarum* used proper fermentation length is expected to generate optimal antioxidant activity which increases the functional properties of the dadih, it can be classified as a functional food. The purpose of the research was to find out the total lactic acid, pH values, soluble protein, and antioxidant activity using different length of fermentation with a combination starter *L. casei* and *L. plantarum*. Complete Random Design with 5 treatments and 4 replicates was used as the experimental design. The treatments given consists of T0 = incubation of dadih for 48 hours, T1 = incubation for 60 hours, T2 = incubation for 72 hours, T3 = incubation during the 84 hours, T4 = incubation for 96 hours. The results showed that long fermentation resulted in the significant changes on the total lactic acid bacteria (LAB), pH values, protein soluble, and antioxidant activity (*P*<0.05). As conclusion, different length of fermentation increased lactic acid bacteria and total antioxidant activity in dadih. Optimum fermentation in 72 hours length of time, this corresponds in order to produce dadih with antioxidant activity and optimal total lactic acid, pH values and protein soluble.

Keywords: Dadih, Long fermentation, Antioxidant activity, Functional food

Introduction
Dadih is one of Buffalo’s milk fermented products originating from the region of West Sumatra (Setiyanto and Zulbardi, 2005; Dharmawan *et al*., 2006). Dadih is ripened for 48 hours without previously warm-up (Manan *et al*., 1999; Setiyanto and Zulbardi, 2005). Dadih’s process is generally still done traditionally, i.e. utilizing microorganisms that exist without any addition of extra starter, same as dadih’s process that is done using bamboo and banana leaves as a cover of bamboo tubes. Dadih as indigenous food of Indonesia has the potential to be developed as good benefits for health as well as yogurt. Although the dadih has the potential to be developed, there are several constraints that are limitations of the amount of buffalo milk and less controlled manufacturing process. The effort to develop dadih by changing the buffalo milk with cow’s milk and modified dadih’s process that can be controlled (Setiyanto and Zulbardi, 2005).

The nutritional characteristics of dadih with a combination of bacteria *L. plantarum*, *L. acidophilus* and *B. bifidum* at temperature of 27 – 33°C for 24 hours, have 3.53% protein; 10.96% fat; 83.15% moisture content; 0.90% ash content; acidity 1.06 – 1.98 ; and the value of pH 3.69 (Taufik, 2004). The fermentation process that occurred in dadih’s process spontaneously is as a result of the interaction of microorganism in bamboo tubes. The fermentation process that occurs can enhance the nutritional value of functional properties, and the formation of secondary metabolites that are beneficial to health.

Fermentation in milk using lactic acid bacteria resulting antioxidant peptides have been identified (Hernandes-Ledesma *et al*., 2005). Antioxidants have functions and capabilities that ward off free radicals, thus preventing the occurrence of degenerative disease, prevented or inhibited the process of premature aging, minimized the occurrence of oxidation process of fats and oils, minimized the occurrence of the damage process foodstuffs, extended the use of a food product, increasing the stability of fat contained in food (Winarsi, 2007). Formation of antioxidant peptides is through the process of fermentation with the breakdown of proteins by enzymes (Akuzawa *et al*., 2009). Using cow's milk curd making bacteria *L. casei* and *L. plantarum* is expected to enhance the taste and value added i.e. functional properties that are beneficial to health. The purpose of this research is evaluate the characteristics of using a combination of cow’s milk with starter *L. casei* and *L. plantarum* on different length of fermentation.

Materials and Method
*Lactobacillus casei* bacteria which is the collection bacteria of Indonesian Agency for Agriculture Research and Development (IAARD) Ministry of Agriculture, Bogor, Indonesia. *Lactobacillus plantarum* FNCC0027 of Gadjah Mada University, Yogyakarta, Indonesia. cow’s milk taken from CV. CITAnasional in Salatiga, Indonesia. All other compounds were reagent grade used to analyze. Spectrophotometry was used for soluble analysis protein
and antioxidant activity, viscometer pipe Ostwald was used for viscosity analysis, pH electric was used for pH value analysis, and other specified compounds.

Preparation of Dadih
The dadih was prepared as method performed or process is carried out in accordance with the research conducted by Usmiati and Setiyanto (2010) with modifications. Next add starter culture L. casei and L. plantarum 1: 1 by as much as 1%. The mixture put into sterile containers, then covered with aluminum foil and incubated at room temperature for 48-96 hours. After incubation complete, the resulted product was cooled immediately at a temperature of 5° C the next step done. Evaluation of the total LAB, pH value, soluble protein and antioxidant activity. Total lactic acid bacteria (LAB) was used the method of Total Plate Count (TPC) (Fardiaz, 1993). The pH value evaluation was used pH electric (Legowo et al., 2009). Evaluation of soluble protein was used the method of lowry (Hammond and Kruger, 1988). Antioxidant activity was conducted as the method that was performed by Mermelstein (2008) with methods 2,2-diphenylpicrylhydrazyl (DPPH).

Experimental design
The study was conducted using Complete Random Design (CRD) with 5 treatments and 4 replications, thus there were 20 unit experiments. Treatments were as follow; T0 = 48 hours, T1 = 60 hours, T2 = 72 hours, T3 = 84 hours, T4 = 96 hours. With combinations L. casei and L. plantarum 1: 1. The parameters observed were total lactic acid bacteria (LAB), pH value, soluble protein, and antioxidant activity. Data were subjected to analysis of variance, and it was continued to Duncan test at a probably level of 5%, when the treatment indicated significant effect. (Gomez and Gomez, 1995). Data were analyzed by using SPSS program.

Results and Discussion
Total lactic acid bacteria (LAB), pH value, and soluble protein
Based on the research showed the difference length of fermentation in dadih was significant on the total of lactic acid bacteria (P<0.05). The fermentation process by L. casei and L. plantarum bacteria that are heterofermentative facultative bacteria changed lactose into lactic acid and other metabolite compounds. Nutrition content in milk can be used by lactic acid bacteria during the length of the fermentation conducted. Nutrition used by lactid acid bacteria were carbohydrate and protein to produce lactic acid and metabolite compounds. This is in accordance with Buckle et al. (1985) carbon and energy sources for almost all of microorganism related to food can be obtained from simple form of glucose and protein as carbin source. L. Plantarum and L. Casei are heterofermentative facultative bacteria. L. Plantarum has wide environment adaptation potential in various application efficiently, produces lactic acid in high concentration, decreases pH and produces ethanol (Plumed-Ferrer 2007; Kawashima et al., 2011).

The result showed that the different length of fermentation in dadih was significant on pH value (P<0.05). Lactic acid and metabolite compounds resulted by lactic acid bacteria decreased pH value to become low, lactic acid in high concentration can decrease pH value to isoelectric point. Different length of fermentation resulted in real impact and the difference among the treatments to pH value of dadih. It is suggested that because the length of fermentation in room temperature and nutrient used influenced the amount of lactic acid production resulted by lactic acid bacteria so that cow’s milk condition became acid and pH milk getting low. According to Miskiyah et al. (2011) milk component that has part in fermentation are lactose and casein that are nutrients for bacteria as source of energy and carbon that are changed by lactic acid bacteria into lactic acid. Based on Akuzawa et al. (2009) fermentation method with inoculating starter bacteria will change lactose in milk into lactic acid during the incubation process that is about 16 – 18 hours.

The result showed that the different length of fermentation in dadih was significant on soluble protein (P<0.05). The increase of soluble protein from 48 hours up to 60 hours assumed that because protein was released in the following ways proteolysis by enzymes derived from bacteria. The decrease of soluble protein rate occurred in 72 hours up to 96 hours. It is assumed that because milk protein source and amino acids in milk was also used by bacteria as source of nutrition, and also because there was protein hydrolysis into peptide in certain length of fermentation. It is assumed that the starter cultures used, L. casei and L. plantarum, are proteolytic bacteria so that they have proteolysis capability to protein in milk. Amount of soluble protein affected by the amount of bacteria associated with proteolytic activity in the presence of protease enzymes that produced by bacteria (Setioningsih et al., 2004). According to Khalid and Marth (1990), L. casei and L. Plantarum obtain proteolitic activity by hidrolisy β-casein in milk. This is in accordance to Tamime and Robinson (1999) about some starter cultures in yogurt that have proteolitic capability will be able to do proteolysis by enzyme hidrolisis from milk protein and as a result, peptida in various size and free amino acid are released, along with the possibility of physical changes in yogurt. Bacteria used in fermented milk product as starter can synthesize amino acid although not completely or just partly. There is also growth simbiosys from a mixed culture, simbiosys can be easily explained. It is an organism that provides useful compounds for other organisms. This is related with the using of amino acids as protein component, where the organism uses protein as metabolism activity for their growth that continuously synthesis amino acids needed by other organisms to grow. According to Misgiyarta (2005) lactic acid production depends on
the type of fermentation and the use of nutrient in milk by lactic acid bacteria. stated that the growth of lactic acid bacteria in milk depends on proteolytic system in hydrolysing protein into peptide and amino acid where proteinase has important role in hidrolis that is to breakdown milk casein (Surono, 2004). According to Yusmarini et al. (2010), the decrease of soluble protein concentration also occured in research for soya milk that was fermented by L. Plantarum for 20 hours. This occurs because bacteria used to hydrolys soy protein during the fermentation process.

Table 1. Total BAL, pH Value, and Dadih soluble Protein with different length of fermentation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average of Total LAB (CFU/ml)</th>
<th>Average of pH Value</th>
<th>Average of Soluble protein (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 (48 hours)</td>
<td>6.4763c</td>
<td>4.50a</td>
<td>12.22cd</td>
</tr>
<tr>
<td>T1 (60 hours)</td>
<td>8.8205b</td>
<td>4.27b</td>
<td>17.46a</td>
</tr>
<tr>
<td>T2 (72 hours)</td>
<td>8.6320b</td>
<td>4.18c</td>
<td>16.23b</td>
</tr>
<tr>
<td>T3 (84 hours)</td>
<td>8.8453b</td>
<td>4.09d</td>
<td>12.16c</td>
</tr>
<tr>
<td>T4 (96 hours)</td>
<td>9.8718a</td>
<td>4.03e</td>
<td>11.60d</td>
</tr>
</tbody>
</table>

*Value bearing different superscript in the same raw indicated significantly different (P<0.05).

Table 2. Dadih antioxidant activity in different length of fermentation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Antioxidant activity mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 (48 hours)</td>
<td>3.0159b</td>
</tr>
<tr>
<td>T1 (60 hours)</td>
<td>4.7843a</td>
</tr>
<tr>
<td>T2 (72 hours)</td>
<td>5.8159a</td>
</tr>
<tr>
<td>T3 (84 hours)</td>
<td>3.5179b</td>
</tr>
<tr>
<td>T4 (96 hours)</td>
<td>3.5984b</td>
</tr>
</tbody>
</table>

*Value bearing different superscript in the same raw indicated significantly different (P<0.05)

The result showed that different length of fermentation in dadih was significant for antioxidant activity (P<0.05). Based on the result obtained, dadih with different length of fermentation showed the existence of antioxidant compound, that showed by the capability of dadih in averting DPPH free radical. Antioxidant activity in dadih with different length of fermentation reached the peak in the 72 hours length of fermentation. Activity of L. casei and L. Plantarum in fermentation process is assumed able to produce or increase compound that has antioxidant activity beside producing lactic acid as metabolite product in certain length of time and the bacteria proteolysis role during the fermentation process. According to Akuzawa et al. (2009) The peptide is assumed coming from milk protein breakdown into free peptide and free amino acid. Free peptide and amino acid released are antioxidant peptide and amino acid that have antioxidant capability. It is also said that antioxidant activity can also be found in some types of cheese. Antioxidant activity and antioxidant peptide has been identified in cheese. Antioxidant activity in cheese by ripening process the amount is far higher compared to antioxidant activity in cheese without ripening. Peptides in milk are decomposed into smaller peptides through enzymatic process, substances that are decomposed also have antioxidant activity capability. In addition, antioxidant activity based on antioxidant peptide in cheese is relevant in tea katekin known as component of antioxidant and antioxidant peptide in cheese like carnosin.

Conclusion

Based on the result of the research, it can be concluded that the 72 hour length of fermentation in the making of cow’s milk dadih is the most optimum length of fermentation to increase total of lactic acid bacteria, acidity, pH value, and antioxidant activity in dadih. Different length of fermentation in the making of cow’s milk dadih with L. casei and L. plantarum as starter cultures is expected able to be used to determine the proper length of fermentation in order to optimally produce antioxidant compound in dadih. The optimum length of fermentation is 72 hours, in order to produce dadih with optimum antioxidant activity. However, further research is still needed to find out people acceptance with organoleptic test.

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