The chemical, sensory properties and population of lactic acid bacteria of cangkuk made from beef meat and kepayang endosperm (*Pangium edule* Reinw) at various fermentation times

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Abstract. Cangkuk is a traditional fermented beef from Indonesia, especially Riau Province, as a specific food where the processing varies according to the ingredients, formulation, and incubation times. Kepayang endosperm is one of the materials used which contain organic acids, tannins, and polyphenol. However, research on beef fermented with addition of kepayang endosperm is still rare. This research was carried out to study the effect of fermentation times to fermented beef in terms of chemical properties, sensory test, and the population of lactic acid bacteria (Lab). A completely randomized design was performed with fermentation time as a treatment, namely: 0 day, 7 days, 14 days, 21 days, and 28 days. Data were analyzed statistically by analysis of variance. The result showed that the fermentation times up to 28 days have no significant effect to nutritional content, and populations of Lab, but significantly increased sensory properties (color, aroma, texture and taste) and could decreased the pH, total titratable acidity (TTA) and panelist acceptance. It can be concluded that fermentation time up to 28 days in processing of fermented beef could maintain nutritional content, populations of Lab, decrease pH, TTA and have good sensory properties.

1 Introduction

Fermented food products usually have a better nutritional value than the original ingredients. The microbes in fermented products can decompose complex components in food into simpler components, making them easier to digest, and also because these microbes can synthesize some vitamins such as riboflavin, B12, and provitamin A [1]. Traditional fermented food in South East Asia regions is still produced today because the product has good nutritional value, desirable organoleptics, and provides health functionalities [2].

Indonesia has a variety of local wisdom, especially in processed foods of animal origin. One of the traditional foods from Riau Province, exactly in Kuantan Singingi Regency is cangkuk (the local name: cangkuak). Cangkuk is fermented beef which made from beef,

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bamboo shoots, salt, and rice [3]. However, cangkuk also was produced with addition kepayang (*Pangium edule* Reinw) endosperm as main ingredient [4].

Fermented meat has the advantage of being a bioactive source; free amino acids have a unique flavor, do not contain pathogenic bacteria, and have better sensory properties [5]. One of the efforts made for traditional preservation is using kepayang endosperm. Kepayang fruit, usually called kluwak or picung, is one of the flora germplasms that produces fruit that can be consumed and has the potential to be used as medicine and spices. According to Sari and Suhartati [6], kepayang fruit can be used as a preservative because it contains a wide variety of chemicals, such as organic acids, tannins, and other compounds. Phytochemical compounds (tannins and flavonoids) can control the proliferation of bacteria in fish and meat, such as *Pseudomonas aeruginosa, Escherichia coli*, and *Staphylococcus aureus* [7].

It has been reported that kepayang endosperm extract is able to inhibit microbial growth in ground beef [8]. The composition of 4% kepayang endosperm and 2% NaCl can preserve skipjack tuna for three days of storage at room temperature [9]. Fresh kepayang endosperm extract effectively inhibits the growth of pathogenic bacteria found in food such as *Bacillus* sp, *Salmonella* sp*, Eschericia* sp [10].

Similar research on Bekamal (Banyuwangi traditional fermented meat) which various fermentation processed (7, 14, 21 days) provides lactic acid bacteria in the product [11], however the organoleptic and chemical properties have not been reported. The previous research was conducted by Wahyudi [12], cangkuk produced with kepayang endosperm at concentration 75%-125% and fermentation times of 0 day, 7 days, and 14 days was able to increase titratable acidity, decrease pH and maintain the protein content. The hypothesis of this research was the extending the fermentation time will affect the nutritional value, organoleptics, and populations of Lab. Therefore, this study to extend the fermentation time up to 28 days. This research was carried out to study the effect of fermentation times to beef fermented in terms of chemical properties, sensory test, and the population of lactic acid bacteria.

2 Methods

2.1 Materials

The ingredients for making cangkuk are part of leg from beef, kepayang endosperm (*Pangium edule* Reinw), cooked rice, natrium chloride, and distilled water. Chemical agents consists of HCl, K_3SO_4, $MgSO_4$, NaOH, H_3BO_4, phenolptalien, CCl_4, 1% w/v hydrochloric acid solution, natrium hydroxide solution, pH 4 and pH 7 buffers solution. Medium MRS and 0,9% NaCl solution for lactic acid bacteria analysis, and mineral water for sensory analysis. All of chemical reagent for chemical analysis were analytical grade.

2.2 Research design

The experimental design was completely randomized design. The treatment for this research was fermentation time in processing cangkuk, which consists of 0 days, 7 days, 14 days, 21 days, and 28 days. All the treatments were repeated three times.

2.3 Research stage

The research stage consists of three levels. The first was preparation of kepayang endosperm and fresh beef. The second was processing of cangkuk based on the various fermentation

time. The third was analysis of observed variables, namely: chemical composition, sensory properties, and population of Lab. The complete of each stage is as follows.

2.3.1 Preparation of beef meat and kepayang endosperm

Preparation of beef meat was initial 4,500 g of fresh beef meat was obtained from Animal slaughtering house Pekanbaru City. The meat was delivered to laboratory in cold condition after placed in the ice box. The meat was cleaned using distilled water and the fat, connective tissue were removed from meat. Then, meat was cut in to the size of 5 x 5 x 2 cm^3. They were then weighed according to treatment (300 g per sample treatment).

The kepayang fruit is washed and cut in half to get the seeds. Then the mucus attached to the seeds is removed by washing them in the clean water. Kepayang seeds are split in half to get the endosperm (seed contents) by scraping them with a knife and spoon. Then the kepayang endosperm is sliced into small pieces and weighed 300 g per sample treatment).

2.3.2 Production of the fermented meat (cangkuk)

Production of the cangkuk according to Mirdhayati [13] with slight modifications (fermentation media and incubation time). Fermented meat was initially prepared as 300 g of small cuts of beef meat for each experimental unit. Then, 300 g of kepayang endosperm were prepared. Then, both meat and kepayang seed were put into each of the polypropylene plastic boxes based on their treatments. Meat is mixed with kepayang endosperm, salt, and rice. Salt and rice were added at 1% of the total weight of beef meat and kepayang endosperm. All of the ingredients were mixed homogeneously and then covered tightly. The fermentation process was in anaerobic conditions, dark room, at room temperature (26–27ºC).

2.3.3 Observed variables

The observed variables were chemical composition : protein and fat [14-15], moisture and ash content [16], pH [17], and total titratable acidity [18]. The sensory properties covered hedonic and hedonic quality test in terms of color, taste, aroma, and texture [19]. Hedonic test using 70 untrained panelists with hedonic scale 1-5. Analysis of population of lactic acid bacteria [20].

2.4 Data analysis

Data on the chemical properties, sensory properties, and population of lactic acid bacteria were presented as the mean values and standard deviation. Data was statistically analyzed using the analysis of variance. When the treatments were significant for parameters, the Duncan Multiple Range Test (DMRT) was used to determine the difference between the samples.

3 Result and discussion

3.1 Chemical properties

The chemical properties of cangkuk made from beef and kepayang endosperm at fermentation time of 0 to 28 days is shown in Table 1. Analysis of variance showed that the fermentation time did not significantly affect the chemical composition, such as protein, fat,

moisture and ash of fermented beef. This result showed that fermenting beef with kepayang endosperm for 0–28 days can maintain the chemical composition of beef. This is because kepayang endosperm contains natural preservatives that can inhibit the growth of spoilage bacteria in meat. It has been reported that khaulmogric acid $(CH)_{12}COOH$, hydrocarbic acid $(CH_2)_{10}COOH$, gorlic acid $(CH_2)_6CHCH(CH_2)_4COOH$, and tannins in kepayang endosperm are antimicrobial agents, inhibit the spoilage of fresh meat, and can maintain the moisture of fermented meat [21]. According to Yohar [22], kepayang endosperm is used by the community as a natural fish preservative agent because it contains antibacterial components such as cyanide acid (HCN) and tannin. These HCN and tannin components can inactivate putrefactive bacteria such as *Pseudomonas aeruginosa, Escherichia coli*, and *Staphylococcus aureus* [23]. The moisture content of fermented beef remains constant because, during the fermentation process of meat with kepayang endosperm, the meat was placed in a tightly closed container so that the evaporation process did not occur [24].

Table 1. The chemical properties of cangkuk made from beef and kepayang endosperm.

Fermentation time (day)	Protein (%)	Fat (%)	Moisture (%)	Ash (%)	pH	Total titratable acidity (%)
T1 (0)	28.38±1.09	1,16±0,30	73.86±2.27	1.70±0.63	5.46 ± 0.05^{a}	$4.76±0.47^{a}$
T2 (7)	25.62±3.10	2.97±1.29	69.69±1.64	1.03±0.57	4.57 ± 0.08^{c}	$3.34±1.87^{ab}$
T3 (14)	27.20±3.61	2.16±0.77	65.74±3.02	1.18±0.03	4.35 ± 0.03^{c}	$1.05±0.91^{c}$
T4 (21)	29.13±2.33	2.47±2.14	69.79±7.68	2.02±0.92	4.90 ± 0.09^{b}	$0.71±0.42^{c}$
T5 (28)	28.61±2.64	1.48±1.71	69.27±1.30	1.31±0.20	4.94 ± 0.18^{b}	$2.07±0.13^{bc}$

Note : different superscripts in the same column indicate very significant differences ($P< 0.01$).

The ash content of fermented beef remains constant between incubation times 0, 7, 14, 21, and 28 days because the minerals in the meat during the fermentation process do not decrease. Fermentation causes the minerals bound in the meat matrix to be easily absorbed, so that fermentation can maintain the mineral levels of the meat and increase the bioavailability of minerals in the meat [25]. The fat content of fermented beef remains constant between incubation times of 0, 7, 14, 21, and 28 days. Organic acid and phenolic compounds in the kepayang endosperm during the fermentation process can inhibit peroxidation or fat breakdown [26].

Fat in the kepayang endosperm, when fermented, will produce unsaturated cyclic fatty acids, namely hydrocarpic acid ($C_{16}H_{28}O_2$) and khaulmogric acid ($C_{18}H_{32}O_2$) [21]. It has been reported that tannins and flavonoids are among the phenolic compounds with antibacterial properties found in fermented kepayang endosperm. Tannins and flavonoids can fight meat spoilage bacteria, such as *Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus* [8].

In term of pH, the fermentation time had a significant effect on decreasing the pH of fermented beef ($P<0.01$). The highest pH value was found in the 0 day treatment, namely 5.46, and the lowest pH value was found in the 7 days and 14 days treatment, namely 4.35-4.57. According pH value, cangkuk is categorized in low acid food. This is due to the occurrence of chemical reactions as well as the length of the fermentation process and the water content in it, which have the effect of reducing the pH of fermented beef. When beef was fermented with kepayang endosperm anaerobically, it produces low levels of lactic acid bacteria, causing the anaerobic glycolysis process to occur. At that time, there is a change in the endogenous enzyme, which becomes active, causing the meat that has been fermented for 28 days to become tender and the lactic acid to accumulate, causing a decrease in pH. This research is in line with [12], research on the chemical characteristics of fermented beef with kepayang endosperm (*Pangium edule* Reinw) at different concentrations and fermentation times, showing that different fermentation times have an effect on reducing the pH of fermented beef with results obtained at 0 days. namely 5.18, and decreased on the 14 days,

namely 4.23. pH of fermented beef in this research were higher than pH of Chinese fermented food product, sour meat, ranging from 4.04 – 4.87 [27].

The fermentation time showed a significant effect on decreasing the total titratable acidity of fermented beef (P<0.01). Total titratable acidity describes the amount of lactic acid produced during the fermentation process. Lactic acid of fermented beef in this research was lower than lactic acid Chinese fermented pork product, sour meat, ranging from 6.4-51.4 % [27]. The total of titratable acidity is closely related to the number of lactic acid bacteria (Table 5). As the fermentation time increases, the total of titratable acidity decreases as a result of the number of lactic acid bacteria remaining constant up to an incubation time of 28 days. The presence of organic acids in the endosperm effectively inhibits putrefactive bacteria and can control the number of lactic acid bacteria.

3.2 Sensory properties

The appearance of cangkuk produced from beef and kepayang endosperm at fermentation time of 0 to 28 days is shown in Figure 1. Sensory analysis of fermented beef was shown in Table 2, Table 3, Table 4, and Table 5.

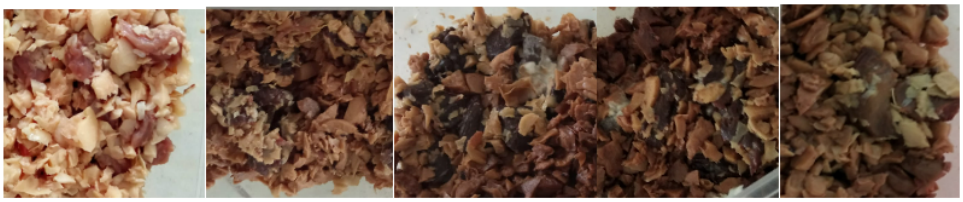

Fig. 1. Beef fermented with addition kepayang endosperm.

Table 2. The color of cangkuk made from beef and kepayang endosperm.

Fermentation time (day)	Hedonic quality score	Description	Hedonic score	Description
T1 (0)	1.45 ± 0.50^{a}	pale pink	$2.87 \pm 0{,}79^{a}$	Dislike
T2 (7)	3.44 ± 0.69^{b}	greyish pink	$2.97 \pm 0{,}68^{a}$	Dislike
T3 (14)	4.08 ± 0.61^{c}	Brownish	$3.27 \pm 0{,}73^{b}$	Neutral
T4 (21)	3.91 ± 0.59^{c}	greyish pink	$3.40 \pm 0{,}63^{b}$	Neutral
T5 (28)	4.18 ± 0.62^{d}	Brownish	$3.43 \pm 0{,}72^{b}$	Neutral

Note : different superscripts in the same column indicate very significant differences (P< 0.01)

According Table 2, fermentation time had a significant effect on increasing the color of fermented beef (P<0.01) in hedonic quality test. As seen in Fig 1 and Table 2, 0-day treatment produces a pale pink color, which is caused by beef pigment (myoglobin) being denatured due to organic acids in the kepayang endosperm. At 7 days of incubation, the color of the flesh becomes pinkish gray, which is caused by the increasing amount of denatured pigment. After 14 days of incubation, it produces a brownish color that is the same as the color of the kepayang endosperm. This brownish color is caused by the tannin content in kepayang. Increasing fermentation time causes tannins to change the color of beef to brownish [28]. In terms of hedonic test, the fermentation time had a significant effect on increasing the hedonic score of color of fermented beef (P<0.01), from dislike (0-7 days) became neutral at 14, 21, and 28 days. This research is in line with the research results of Aldona [29], the organoleptic test of fermented Bali beef (cangkuk) with different incubation times obtained the highest color at 28 days (brownish red criteria), and the lowest at 7 days of treatment (red criteria).

Increasing the fermentation time up to 28 days had a significant effect on increasing the aroma of fermented beef (P<0.01) in hedonic quality test. As seen in Table 3, 0-day treatment produces a medium woody aroma, little sour which is produced by organic compound in the flesh kepayang endosperm such tannin, HCN. At 7 days of incubation, the woody aroma increased which is caused by the increasing amount of tannin absorbed by the meat during the incubation process. This phenomenon also occurred until 21 days incubation time. The highest aroma score occurred at 28 days of storage, where a stronger sour aroma was detected due to the activity of lactic acid bacteria. In terms of hedonic test, the fermentation time had a significant effect on increasing the hedonic score of aroma of fermented beef (P<0.01), from dislike (0-day) became neutral at 7, 14, 21, and 28 days.

In this research, increasing the fermentation time up to 28 days had a significant effect on increasing the texture of fermented beef (P<0.01) in hedonic quality test. As seen in Table 4, 0-7 days treatment produces a little bit tender of texture of fermented beef, and at 14 day of incubation time can produce fermented beef with tender in texture. This phenomenon also occurred at 21 days and 28 days of incubation time. This is caused by the activity of endogenous enzymes in the meat, where at the beginning of fermentation at pH 5.4, the myofibril protein degradation process is still slow, so the meat is rather tender. The length of the meat fermentation process affects the texture of the meat. With increasing fermentation time and the presence of organic acids from kepayang, the pH decreases so that endogenous enzymes (cathepsin and calpain) in beef are active in degrading myofibrils and disruption of sarcomere structure in the meat, so that the meat becomes tender [30].

Table 3. The aroma of cangkuk made from beef and kepayang endosperm.

Fermentation time (day)	Hedonic quality score	Description	Hedonic score	Description
T1 (0)	2.27 ± 0.75^{a}	Medium woody aroma, little sour aroma	2.72 ± 0.79^{a}	Dislike
T2 (7)	3.19 ± 0.71^{b}	Strong woody aroma, little sour aroma	3.02 ± 0.65^{b}	Neutral
T3 (14)	3.82 ± 0.79^{c}	Strong woody aroma, little sour aroma	3.14 ± 0.85^{b}	Neutral
T4 (21)	3.70 ± 0.80^{c}	Strong woody aroma, little sour aroma	3.16 ± 0.62^{b}	Neutral
T5 (28)	4.03 ± 0.91^{d}	Strong woody aroma, sour aroma	3.29 ± 0.68^{b}	Neutral

Note : different superscripts in the same column indicate very significant differences (P< 0.01).

Table 4. The texture of cangkuk made from beef and kepayang endosperm.

Fermentation time (day)	Hedonic quality score	Description	Hedonic score	Description
T1 (0)	2.43 ± 0.80^{a}	little bit tender	3.06 ± 0.61^{a}	Neutral
T2 (7)	2.75 ± 0.87^{a}	little bit tender	3.14 ± 0.56^{a}	Neutral
T3 (14)	3.10 ± 0.95^{b}	Tender	3.24 ± 0.69^{a}	Neutral
T4 (21)	3.37 ± 0.98^{bc}	Tender	3.08 ± 0.77^{a}	Neutral
T5 (28)	3.58 ± 0.71^{c}	Tender	3.43 ± 0.54^{b}	Neutral

Note : different superscripts in the same column indicate very significant differences (P< 0.01).

Table 5. The taste of cangkuk made from beef and kepayang endosperm.

Fermentation time (day)	Hedonic quality score	Description	Hedonic score	Description
T1 (0)	3.12 ± 0.54^{a}	Little bit sour	3.01 ± 0.55^{a}	Neutral
T2 (7)	3.27 ± 0.75^{a}	Little bit sour	3.09 ± 0.69^{ab}	Neutral
T3 (14)	3.82 ± 0.81^{b}	Little bit sour	3.33 ± 0.62^{bc}	Neutral
T4 (21)	3.71 ± 0.99^{bc}	Little bit sour	3.44 ± 0.81^{c}	Neutral
T5 (28)	4.04 ± 0.83^{c}	Sour, Little bit savory	3.61 ± 0.57^{d}	Neutral

Note : different superscripts in the same column indicate very significant differences ($P< 0.01$).

In term of taste of fermented beef, the fermentation time had a significant effect on increasing the taste ($P<0.01$) in hedonic quality test. As seen in Table 5, 0-7 days treatment produces a little bit sour of taste of fermented beef, and at 14-21 days of fermentation time can increase score of taste, and at 28 days of fermentation time produce fermented beef with sour in taste and identify little bit savory. In term of hedonic test, panelist give the respond neutral to texture and taste of fermented beef. The panelists only rated neutral on the attributes of color, aroma, texture, and taste of cangkuk because they were not familiar with fermented beef. When compared with Chinese traditional fermented pork (Suan rou), fresh streaky pork was cured with cinnamon, spices, glutinous rice, salt and then fermented in anaerobic condition during 30 days, produced fermented meat with high overall acceptability, good in taste, appearance, colour, flavour [31]. The addition of rice and salt in the processing of fermented meat also contribute in taste. The taste of a food can derived from the food ingredient itself and other ingredients in the product that are added to it [32].

3.3 Population of lactic acid bacteria

The population of lactic acid bacteria (Lab) was shown in Table 6. In this research, fermentation time up to 28 days did not significantly affect the population of lactic acid bacteria. There is no increase in the microbial population between the treatments. The flesh of the kepayang endosperm has a high content of cyanide acid (HCN) and fatty acids, namely khaulmogric acid, hydrocarpic acid, and gorlic acid, and also has a high content of alkaloids, which are reported to have strong antibacterial compounds. Therefore, these compounds are thought to be able to inhibit the growth of lactic acid bacteria so that the population of lactic acid bacteria remains low for 28 days of fermentation. The kepayang endosperm contain antimicrobial compounds that can inhibit bacterial growth [33]. Furthermore, fermenting beef with kepayang endosperm can produce substances that have antibacterial properties. The low population of lactic acid bacteria is also caused by the high concentration of kepayang endosperm flesh, with ratio between beef and kepayang endosperm used was 1:1. Therefore, kepayang endosperm flesh's chemical components are highly effective at preventing the growth of lactic acid bacteria.

The findings of this study demonstrate that the chemicals in kepayang endosperm play a function in meat preservation because they can keep the chemical composition and the quantity of lactic acid bacteria consistent. In this experiment, lactic acid bacteria could only survive and not well growth along fermentation process was prolonged up to 28 days.

Table 6. Population of lab of cangkuk.

Fermentation time (day)	Total of lactic acid bacteria (cfu/g)
T1 (0)	$7{,}58 \times 10^1$
T2 (7)	$2{,}34 \times 10^2$
T3 (14)	$1{,}07 \times 10^2$
T4 (21)	$1{,}02 \times 10^2$
T5 (28)	$1{,}20 \times 10^1$

4 Conclusion

Cangkuk is traditional fermented beef can produced with addition of kepayang endosperm which fermentation time up to 28 days provide good nutritional value, low acid food, low population of lactic acid bacteria (1.20×10^1 to 2.34×10^2 cfu), have a good hedonic quality : brownish color (4.18 ± 0.62), very strong wood aroma, sour (4.03 ± 0.91), tender texture (3.58 ± 0.71), and sour taste (4.04 ± 0.83). The next research challenge is how to increase consumer acceptance of cangkuk.

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