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Analysis of the Microbiological Quality and Organoleptic Characteristics of *Se'i* Beef Typical Timor East Nusa Tenggara Indonesia on the Storage Period and Lime Juice Concentration Different

Research Article

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Abstract

Se'i beef is a typical food from Timor island which is very popular with most people, but this meat is easily damaged, therefore special treatment is needed to extend the shelf life. The purpose of this study was to examine the effect of the concentration differences of lime juice and storage period and the interaction of these two factors on the microbiological quality of *se'i* beef. This study uses a factorial design consist of 2 factors, namely the concentration of lime juice and storage period. The lime juice factor consists of 3 concentrations, namely 0 mL/300 g, 125 mL/300 g, and 250 mL/300 g. The storage period factor consists of 0, 2, 4, and 6 days. The data collected consisted of the number of colonies, aroma, savor, tenderness, and color of the *se'i* beef. Data on the number of colonies were analyzed by Two Way ANOVA at a significance level of 5%. Organoleptic data were analyzed using descriptive statistics. The results showed that the concentration of lime juice affected the microbiology quality of *se'i* beef, but no affected of the storage period on the bacterial growth and no interaction between these two factors. The results of organoleptic analysts showed that the concentration of lime juice and storage period affected the aroma, savor, and color of meat but the tenderness of the *se'i* beef was not influenced by the concentration of lime juice.Concentration of lime juice affected the microbiological quality of *se'i* beef.

Keywords: *se'i* Beef; Lime Juice; Storage Period; Organoleptic; Bacterial Colonies.

Introduction

Beef is a source of animal protein that is very popular with the community. The people's penchant for beef is shown by the increasing demand for beef from year to year. The national demand for beef cattle in 2019 is 376,360 tonnes (2.9 million head), increasing in 2020 to 388,583 tonnes (2.12 million heads) [1].

The beef is processed in traditional or modern in a variety of products to provide opportunities for the consumer to choose according to their tastes. One of the beef products that is traditionally processed is *se'i* beef (a local name for Timorese Ethnicity, Indonesia). *Se'i* beef is one of the culinary icons at Kupang City, East of Nusa Tenggara Indonesia. *Se'i* beef processing is done through fumigation using *Kesambi* timber (*Schleicheraoleosa* Merr 1917=*S.oleosa*). Raza [2] states that aging *se'i* is a typical Timor smoked meat that is smoked using S. oleosa wood. The purpose of smoking is to improve taste, aroma, and shelf life. The increase in shelf life is caused by the presence of bioactive compounds contained in the bark of *S. oleosa*. *Situmeang* et al. [3], Hanifah and Kiptiyah [4], state that in *S. oleosa* leaf extract there are secondary metabolites of alkaloids, flavonoids, steroids, phenolics, and tannins. Phenolic compounds are one of the active compounds as toxic compounds for microbes [5] and phenolic compounds derived from wood smoke will increase the aroma of *se'i* beef [6]. The presence of these bioactive compounds inhibits microbial activity, thereby extending the shelf life of se'i beef.

The facts show that *se'i* beef processing is done traditionally in an unhygienic place, so there is a very big chance of contamination. Meat contaminated with microbes that exceed the threshold will become slimy, moldy, decrease storage capacity, smell bad, taste bad, and cause health problems when consumed [7]. This is caused by the development of bacteria in the meat that has

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exceeded the threshold. The maximum limit of microbial contamination in raw and processed meat is 102 - 105 colonies/gram [8]. Food that has been contaminated by microbes exceeding the threshold will be toxic to humans because, in addition to destroying foodstuffs, bacteria can produce toxins, both exotoxins, and endotoxins.

Se'i beef damage control is carried out in various ways, including by storing it in the refrigerator or by adding preservatives. One of the natural preservatives that can be used is lime juice (*Citrus aurantifolia*). Besides being a preservative, lime juice can add flavor to the meat. Some of the secondary metabolites contained in lime include saponins, flavonoids, essential oils [9-12]. Razak et al. [13] suggest that lime has chemical properties such as essential oils and phenols which are bactericidal.

Another advantage of using lemon juice as a natural preservative is that lime juice contains elements of chemical compounds that are useful such as citric acid, amino acids, glycosides, citric acid, fat, calcium, phosphorus, iron, sulfur, vitamin B1, and vitamin C [14]. Lime juice contains compounds of organic acids such as citric acid, malic acid, lactic acid, and tartaric acid [15]. These chemicals are essential for the human body. The research of Mau [16] shows that the variation of seasoning and storage time at room temperature of *se'i* meat has a very significant effect on *se'i* meat samples using lime and without lime. Growth slower bacteria in the samples using a lemon extract. The application oforange extract and the method of curing affects the color, aroma, tenderness, and pH but does not affect the taste, nitrite residue in *se'i* meat.

This lime juice can be used as a preservative by adding it during curing with the meat before smoking. The purpose of this ripening is so that the lime juice can be absorbed evenly in the meat and can increase the taste and tenderness in the meat, and inhibit the growth of bacteria. In this regard, it is necessary to study the effect of lime juice concentration and the storage period and the interaction of these two factors on the growth of bacterial colonies, taste, tenderness, aroma, and color of *se'i* beef.

Materials and Methods

Research design

Research is using design factorial consist of two factors, namely the concentration of lime juice and storage period. The lime juice factor consists of 3 concentrations, namely 0 mL/300 g, 125 mL/300 g, and 250 mL/300 g. The storage period consists of 4 levels, namely 0 days, 2 days, 4 days, and 6 days.

Se'i beef making

The beef used is 2 kg. The meat is cleaned, separated from the fat and connective tissue, and washed. The meat is sliced lengthwise, after which the meat is seasoned, namely 175 g of table salt (NaCl) and 100 g of granulated sugar then mixed until homogeneous. After that, the meat is divided into 3 parts.

Juice lime making

Lime is weighed as much as 2 kg, washed thoroughly then sliced and squeezed using an orange juice squeezer. The results of the lime juice are filtered and the juice is taken. Beef marinated in the lime juice by the treatment, ie 125 ml/300 g meat and 250 mL/300 g meat, and let stand for 8 hours. As a control, beef was only given spices without soaking in lime juice.

Fumigation

After soaking the meat samples in lime juice, the samples of meat and beef without soaking were smoked using *S. oleosa* wood. The meat is placed on a bamboo rack and allowed to stand until the water drips. The smoking drum is filled with wood on the porch and burned until it becomes embers and emits smoke. After the fire emits smoke, beef is placed on a grill rack on a drum with a distance of ± 30 cm between the meat and the coals [17]. The top of the meat is covered with washed *S. oleosa* leaves. During smoking, turn the beef back for 10 - 20 minutes (depending on the heat used) until the meat is half cooked. After smoking, *se'i* beef was stored for 0, 2, 4, and 6 days before data collection on the number of colonies and organoleptic tests.

Calculation of the Colonies Number

Colony counts were calculated using a colony counter to calculate the total number of bacteria per gram of sample. The normal range for the number of colonies on each plate is $30 \leq x \leq 300$, where x is the number of colonies per plate or dilution. The unit used is the Colony Forming Unit (CFU) per gram. Sieuwert et al. [18] stated that the quantitative population analysis method was carried out by calculating the CFU in the dilution series. The steps for planting and calculating the number of colonies are as follows: a. Weighed 10 grams sample *se'i* beef sterile and mashed with a blender that has been in a sterile right. b. Meat samples that have been mashed are transferred to Erlenmeyer 100 mL which contains 90 mL sterile saline solution, then homogenized for one minute. c. Pipette 1 mL homogenate into a test tube containing 9 mL sterile physiological salt then shaken for 3 minutes. d. Furthermore, the homogenate dilution is carried out up to 10,000 times or 10-4. e. From 10-4 dilution 1 mL pipettes and transferred to a petri dish containing NA media. Homogenate is spread evenly with a spreader over the surface of the media. f. Planting all combination treatments following procedure a - e.g. All Petri dishes were incubated at 37ºC for 24 hours. h. Colonies that grew on the media were counted using a colony counter.

Organoleptic test

Organoleptic tests were carried out by involving panellists to assess aroma, taste, tenderness and color. The panelists used were semi-trained panelists (who often consume *se'i* beef) as many as 20 people, consist of 10 men and 10 women from Biology Education students of Nusa Cendana University. Each organoleptic given a score of 1-4 with following criteria: (a) aroma (1 = foul smell, 2 = sour smell, 3 = smoke smelled, 4 = smelled tipycal of *Se'i* beef), (b) Taste (1 = not delicious, 2 = rather delicious, 3 = delicious, 4 = delicious typical of Se'i beef), (c) tenderness (1 = not soft, 2 = slightly tender, 3 = soft, 4 = very tender typical of *Se'i* beef) and (d) Color (1 = blackish brown, 2 = brownish red, 3 = pale red, 4 = bright red typical of *Se'i* beef).

Data analysis

An organoleptic test was done by tabulating data from each parameter. The quality category of each parameter is determined based on the total value of all panelists. The number of panelists is 20 people with a quality scale of 1 to 4, then the lowest score is 20, the highest score is 80 and the number of categories is 4. Based on this lowest and highest score, the class width = (*highest score-lowest score*)/(*categories number*) = (80-20)/4=15.

The decision to assign a category for each organoleptic parameter is as follows:

The colony number data obtained from the combination treatment of lime juice and storage period were analyzed using the Two-Way ANOVA test. If the p-value < α at the 5% significance level, then proceed with the Duncan test to find out in more detail which pair of groups are significantly different from each other.

Results and Discussion

***Se'i* beef color:** The results showed that the fresh beef samples had a bright red color. After soaking with lime juice for 12 hours and smoking it with S. oleosa wood, the color of the *Se'i* beef changes as shown in table 2.

Based on table 2, it can be seen that the color of *se'i* beef changes color in the 0 days storage period treatment with lime juice 250 mL/300g. The color of the meat did not change at 0 days of storage period at a concentration of 0 mL/300g and 125 mL/300 g. A drastic change in color to blackish brown occurred on the fourth and sixth days of all volume treatment of lime juice.

The results showed that in the 0 days storage period without giving lime juice, the color of the se'i beef flesh did not change. Likewise, at a storage period of 0 days with 125 mL/300 g of lime juice. This fact illustrates that when the meat is soaked in 125 mL of lime juice, the color of the meat does not change so that when smoked with *S. oleosa* wood it still shows thedistinctive color of se'i beef. Treatment with 125 mL/300 g did not fade the bright red color of the meat. Thus, when smoking is carried out, the carbonyl groups contained in the smoke react with the protein to produce a distinctive color of *se'i* beef. When administering 250 mL of lime juice, the color of the *se'i* beef changes to plate red after being given smoking. Rahmawati [19] stated that lemon juice contains citrate acid and Anon[20]stated that lemon juice also contains vitamin C (ascorbic acid) at levels of 27 mg/100 g fruit. Treatment with 125 mL/300 g did not change the original color of the beef (bright red). Ermawati *et al.* [21] stated that ascorbate compounds can also be used as reducing agents that can reduce metmyoglobin to myoglobin and then reacts with nitric oxide to produce nitric oxide myoglobin which is bright red. While the treatment with lime juice 250 mL/300 g, the content of ascorbic acid in excessive amounts so that it can bleach the original color of the beef and change to plate red when smoked with *S. oleosa* wood. Thohari et al. [22] explained that phenolic elements and organic acids are the two elements that are most commonly attached to smoking products and produce the distinctive color of meat.

The aroma of *se'i* beef: Changes in the aroma of se'i meat based on the volume of lime juice and the storage period can be seen in the following table 3.

Table 1. Criteria for categorizing organoleptic parameters.

No.	Score range	Category	Decision
1	20 – 34	Not good	If the total score of all the panelists falls within one of the score ranges, the taste category is equivalent to the presence of that total score.
2	35 – 49	Good enough	
3	50 – 64	Good	
4	65 – 80	Good typical of se'i beef	

Table 2. A score of color change in *se'i* beef.

No.	Storage Period	Se'i beef Color Change		
		0 mL/300 g	125 mL/300 g	250 mL/300 g
1	0 days	78 (brts)	75 (brts)	55 (pr)
2	2 days	42 (br)	40 (br)	32 (bb)
3	4 days	22 (bb)	21 (bb)	22 (bb)
4	6 days	20 (bb)	20 (bb)	20 (bb)

Note: brts = bright red typical of se'i beef, pr = pale red, br = brownish red, bb = brown blackish

Table 3. Scores of change in the aroma of *se'i* beef.

No.	Storage Period	Changes in the aroma of *se'i* beef		
		0 mL/300 g	125 mL/300 g	250 mL/300 g
1	0 days	77 (tss)	76 (tss)	71 (tss)
2	2 days	71 (tss)	72 (tss)	70 (tss)
3	4 days	52 (sms)	45 (ss)	44 (ss)
4	6 days	42 (ss)	42 (ss)	41 (ss)

Note: ss = sour smell, sms = smoky smell, tss = smell typical of *se'i* beef.

Table 3 shows that the distinctive aroma is relatively stable in storage 0 days and 2 days with volume lime juice 0 mL and 125 mL. While on a 4 days storage period at a volume of lime juice 125 mL and 250 mL, and 6 days of the storage period in all treatments lime volume, distinctive aroma of meat *se'i* is smelled sour or are in the category of sour smell.

Based on these data, can be stated that the treatment 0 days and 2 days without lime juice and lime juice 125 mL and 250 mL/300 g to produce smells like the original scent of meat *se'i* beef. Starting on the fourth day, the smell of *se'i* meat begins to change. The distinctive aroma of se'i beef changes to smoky smells then becomes sour. This change in aroma occurs because on the fourth day the ability of lime and S. oleosa smoke as preservatives begins to decrease inprotecting the decay process by microbes. Microbial metabolism produces organic acids, giving rise to a sour smell in *se'i* beef. Van Berkelet al. [23] stated that when smoking with S. oleosa wood, smoke particles absorbed by the meat inhibit bacterial growth, limit lipid oxidation and increase sensory characteristics (aroma, taste, color, and tenderness). The chemical compounds contained in smoke have a bacteriostatic, bactericidal effect and inhibit fat oxidation [22]. The aroma of meat changes due to precursors that enter through water and fat in the release of volatile substances contained in the meat that comes out during the ripening process [24].

The taste of *se'i* beef: The change in taste of *se'i* beef that has been treated based on the volume of lime juice and the storage period can be seen in table 4.

Based on table 4, it can be stated that changes in the taste of se'i beef occur in the storage period of 6 days. In this storage period for each volume of lime juice, the taste of *se'i* beef is in the rather delicious category. In the storage period of 4 days, each volume shows the distinctive flavor of the se'i beef changes to be delicious.

Based on the data in table 4, it can be stated that in the 2-days storage period, the resulting taste is typical of *se'i* beef. At the 0 days storage period, the quality of the taste was slightly below the 2 day storage period with the administration of 125 mL/300 g and 250 mL/300 g of lime juice. In the 0 days storage period without lime juice, the taste of *se'i* beef is solely determined by the smoke of S. oleosa. Meanwhile, at 0 days with lime juice 125 mL/300 g and 250 mL/300 g of meat, the meat has a distinctive taste of *se'i* beef. The taste of *se'i* beef is strongly influenced by the citric acid in the lime juice. Ovelando et al. [25], stated that citric acid is a compound that affects the taste and improves the taste of a product. Meanwhile, in the treatment 0 days without lime juice, taste or flavor is strongly influenced by the presence of volatile products such as aldehydes, ketones, acids alcohols, and hydrocarbons are derived from compounds found in *S. oleosa* smoke and fat decomposition. Meanwhile, Malelak et al. [26] stated that giving lime extract can change the taste attributes of *se'i* beef. Thus the taste of *se'i* beef is strongly influenced by the breakdown of fat, phenolic compounds in *S. oleosa* smoke, and organic acids in lime juice.

The tenderness of *se'i* beef: The change in tenderness in *se'i* meat that has been treated based on the volume of lime juice and storage time can be seen in the table below.

Based on table 5, it can be seen that the meat tenderness changes in the storage period of 4 days and 6 days. In the storage period of 4 days, there was a change in the tenderness of *se'i* beef in each volume of lime juice, and the storage period of 6 days, the *se'i* beef was in the not soft category.

Meat tenderness is a parameter in determining the quality of sen-

Table 4. A score of changes in flavors of *se'i* beef.

No	Storage Period	Changes in the taste of *se'i* beef		
		0 mL/300 g	125 mL/300 g	250 mL/300 g
1	0 days	63 (d)	66 (dts)	66 (dts)
2	2 days	73 (dts)	70 (dts)	70 (dts)
3	4 days	51 (d)	53 (d)	50 (d)
4	6 days	43 (rd)	44 (rd)	45 (rd)

Note: rd = rather delicious, d = delicious, dts = delicious typical of *se'i* beef.

Table 5. A score of changes in the tenderness of *se'i* beef.

No	Storage Period	The change in the tenderness of *se'i* beef		
		0 mL/300 g	125 mL/300 g	250 mL/300 g
1	0 days	71 (vtt)	72 (vtt)	75 (vtt)
2	2 days	67 (vtt)	65 (vtt)	65 (vtt)
3	4 days	49 (lt)	48 (lt)	47 (lt)
4	6 days	25 (ns)	24 (ns)	26 (ns)

Note: ns = not soft, lt = less tender, vtt = very tender typical of *se'i* beef.

sory-tested meat. Consumers prefer to consume se'i beef which is tender compared to hard *se'i* beef. However, the tenderness of the meat is still less controlled by the beef industry [27]. Tenderness is a factor that affects product quality, especially with consumer tastes, and affects general acceptance. The meat tenderness can be determined by measuring its braking power. The lower the breaking value, the more tender the meat will be [28]. At the 0-day storage control, 125 mL and 250 mL of lime juice, meat tenderness tended to increase. Likewise, in the 2-day storage period, *se'i* beef tenderness is still in the very tender typical category of *se'i* beef. The tenderness of *se'i* beef is strongly influenced by citric acid and ascorbic acid in the lime juice. Purnamasari et al. [29] suggested that meat tenderness can be obtained through immersion in acids such as fruit juice. However, along with the longer the storage period in lime juice, the meat tenderness decreased. As explained by Purnamasari et al. [30] stated that heating the meat after soaking in acid, the connective tissue becomes softer, however in the end the myofibril protein will coagulate and become tougher. Purnamasari and Aulawi [29] emphasized that soaking beef in pineapple juice improves taste, but the meat shrinks due to reduced binding capacity to water. This statement illustrates that the longer the meat is soaked in acid, the less tenderness of the meat is because the protein's binding capacity to water decreases. Tiofani [31] explains that the use of various concentrations of lime juice affects the water-holding capacity and tenderness of the laying hens. Mickalek et al. [32] showed that the use of citric acid found in a lime extract with a concentration of 5-10% can provide good taste and increase meat tenderness.

Based on the results of this study, it is argued that the color, aroma, taste, and texture of *se'i* beef are strongly influenced by the smoke of *S. oleosa* and lime juice. The carbonyl groups contained in smoke react with proteins, fats, and carbohydrates in the meat to produce a distinctive color, aroma, taste, and texture. The main carbonyl component in smoke which plays an important role is phenol. This component can act as an antioxidant and produce a brown color when it reacts with oxygen in the air. Meanwhile, phenols that play a role in smell and taste are guaiacol, 4-methyl guaiacol, 2,6-dimethoxy phenol [33-36].

Bacterial colony: This study tested the effect of lime juice (Citrus aurantifolia) and storage period on the microbiological quality of *se'i* beef. The following are the results of the colony calculations.

Based on table 6, it can be seen that there are differences in the number of colonies that grow on *se'i* beef at each volume of lime juice at each storage period. In Table 6, it is clear that the number of bacterial colonies decreases along with the increase in the volume of lime juice. Conversely, the number of bacterial colonies increases frequently by increasing the storage period. Based on the data on the number of bacterial colonies, the following is a graphic image of the average number of colonies in the combination treatment as follows:

Based on Figure 1, it can be seen that in the storage period of 0 days to the second day, the second day to the fourth day, and the fourth day to the sixth day without giving lime juice there was an increase in the number of colonies, respectively 16.55%, 1.76%, and 5.23%. In the storage period of 0 days, 2 days, 4 days, and 6 days of giving 125 mL/300 g of lime juice, there was an increase in the number of bacterial colonies respectively by 5.08%, 3.99%, and 6.67% and in the 2-day storage period, giving lime juice 250 mL/300 g can reduce the number of colonies as much as 62.54%. However, the number of bacterial colonies increased in the storage period of 4 days and 6 days, respectively 16.22% and 16.55%. In the 0 day storage period, giving 125 mL/300 g and 250 mL/300 g of lime juice was able to reduce the number of colonies by 47.93% and 60.73% from the control 0 mL/300 g beef. In the 2-day storage period, treatment with 125 mL/300 g of lime juice was able to reduce the number of colonies by 53.06% and 121.97% of the total number of control colonies. In the 4-day storage period, soaking with 125 mL/300 g of lime juice and 300 mL/300 g of meat, was able to reduce the number of colonies, respectively 52.03% and 117.29% and in the storage period of 6 days in the treatment the same, able to reduce the number of colonies, respectively 51.38% and 114.07%.

Based on the data in Table 6 and Figure 1, it can be argued that treatment with lime juice can reduce the number of bacterial colonies. The ability of lime juice in reducing the number of colonies because lime juice contains bioactive compounds that act as antibacterials. In control without immersion in lime juice, colony growth continued to increase with the storageperiod. From the aspect of the number of colonies, it can be seen that the number of colonies in all combinations of treatment and control has not exceeded the standards set by BPOM [8] . Based on the number of colonies, it can be seen that the number of colonies in all combinations of treatment and control has exceeded the standard set out by BPOM [8]. However, increasing the concentration of lime juice was able to reduce the number of colonies in each storage period. In the treatment of lime juice 250 mL/300 g in the storage period of 2 days, 4 days, and 6 days, there was a decrease in the number of bacterial colonies as much as 121.97%, 117.29%, and

Figure 1. Average bacterial colonies in combination treatment.

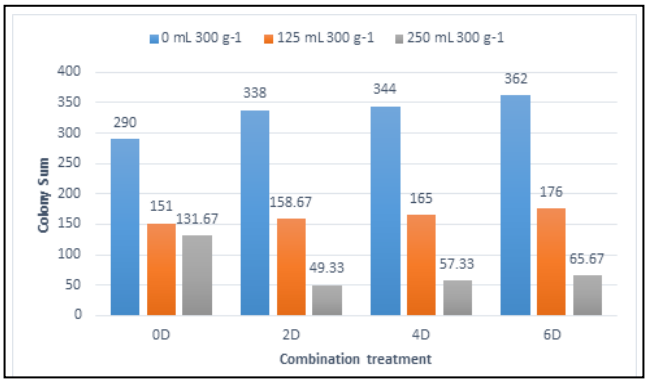

Table 6. Number of colonies in *se'i* beef.

No.	Storage Period	Volume lime (Citrus aurantifolia)		
		0 mL/300 g	125 mL/300 g	250 mL/300 g
1	0 days	298	150	129
		290	156	132
		282	147	134
2	2 days	346	166	53
		339	156	49
		329	154	46
3	4 days	347	171	63
		344	165	56
		341	159	53
4	6 days	368	191	70
		360	170	66
		358	167	61

114.07%, respectively.

In the control without lime juice, the ability to control bacterial growth was solely through the bioactive compounds in the *S. oleosa* smoke. *S. oleosa* contains active compounds which act as antimicrobial, such as alkaloids, flavonoids, phenolics, and tannins [3], terpenoids, flavonoids, phenolic acids, betulin, and betulin acids [37], taraxerone and tricardenic acid [38]. In this treatment, the number of colonies that grew was between 102-105 coloniesg-1. The effectiveness of controlling the number of colonies increased when treated with lime juice with a higher concentration. Bioactive compounds in lime include flavonoids, saponins, and essential oils [39], essential oils and phenols which are bactericidal [13], essential oils and flavonoids as antibacterial agents [14]. Martin et al. [40] explained that the flavonoids and vitamin C contained in C. aurantifolia gave the main contribution to antibacterial activity.

The results of data analysis using Two-Way Anova: The results of the Two-Way ANOVA test of the effect of lime juice concentration at different storage periods on the number of colonies growing on *se'i* beef can be seen in the following ANOVA summary table 7:

Based on the results of the analysis in table 7, it can be argued that lime juice with different concentrations (VG) at each storage period (SP) has a significantly different effect on the growth of bacterial colonies in *se'i* beef. This statement is supported by the results of the analysis which showed that the p-value (0.000) <0.05. Meanwhile, in the storage period, the p-value (0.948)> 0.05, thus it was concluded that the storage period had no significant effect on colony growth in *se'i* beef. The analysis also shows that there is no interaction between the concentration of lime juice and the storage period. This is indicated by the results of the analysis p (0.521)> 0.05. Based on the results of this analysis, further analysis of the effect of lime juice concentration was carried out with the Duncan distance test. The analysis results are shown as follows:

Based on the data in table 8, it can be concluded that the average number of bacterial colonies between treatments is significantly different at the 5% significance level. This means that giving lime juice with different concentrations results in a different number of bacterial colonies. Table 7 shows that the number of bacterial colonies on the control different with the treatment of 125mL/300 g and 250 mL/300 g of *se'i* beef. Likewise, the number of bacterial colonies in the treatment 125 mL/300 g was different from the treatment 250 mL/300 g. The effect of the storage period and the interaction between the storage period and the concentration of lime juice was not carried out a further analysis because there was no significant effect on the number of bacterial colonies.

The effect of lime juice concentration on the number of bacterial colonies is caused by the presence of bioactive compounds that act as antibacterial agents in lime juice. As previously stated that in lime juice there are flavonoids, saponins, essential oils which act as antibacterials[13, 14, 39, 40]. The higher the concentration, the more bioactive compounds will be contained. The higher the bioactive compound content, the higher its ability to control bacterial growth. As shown in Table 6, in the control without lime juice, the number of bacterial colonies was higher than that of 125 mL/300 g and 250 mL/300 g of lime juice. The number of colonies in the treatment 125 mL/300 g lime juice was higher than the 250 mL/300 g. This illustrates that the higher the concentration, the higher the ability of bioactive compounds to reduce bacterial colonies.

Flavonoid antibacterialsinhibit or kill bacteria through the formation of complex compounds with extracellular proteins and dissolved proteins so that they can damage bacterial cell membranes [14]. Flavonoids can damage cell membranes by inhibiting macromolecular synthesis, depolarizing cell membranes, and inhibiting the synthesis of DNA, RNA, and protein [42]. Meanwhile, saponins inhibit bacterial growth through cell leakage mechanisms. Nuria et al. [41] stated that saponins reduce surface tension resulting in increased permeability or leakage of bacterial cells followed by the release of intracellular compounds. Nuraini [43] emphasized that saponins inhibit growth or kill bacteria through interactions with sterol membranes. The main effect of saponins on bacteria is the release of proteins and enzymes from the cell. In the 6-day storage period of each lime juice concentration an increase in the number of bacterial colonies occurred. This is caused by the reduced ability of antibacterials to inhibit or kill bacteria.

Table 7. Summary of Two Way Anova.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	414963.222 a	11	37723.929	44.455	0
Intercept	1309498.778	1	1309498.778	1543.159	0
VG	412005.556	2	20 6002.778	242.761	0
SP	304.333	3	101.444	0.12	0.948
VG * SP	2653.333	6	442.222	0.521	0.787
Error	20366	24	848.583		
Total	1744828	36			
Corrected Total	435329.222	35			
a. R Squared =.953 (Adjusted R Squared =.932)					

Table 8. Differences in the average colony between treatments.

Colony				
Duncan [a, b]				
VG	N	Subset		
		1	2	3
3	12	76.0000		
2	12		162.667	
1	12			333.5000
Sig.		1.000	1.000	1.000

Besides bioactive compounds that act as antibacterials, controlling bacterial growth through changes in pH. In lime juice, there are several organic acids. Prastiwi and Ferdiansyah [44] state that in lime juice there are citric acid, malic acid, and tartaric acid. The presence of this organic acid will lower the pH value. In acidic conditions, the bacteria can survive in a group of bacteria acidophilic, while bacteria neutrophilic and basophilic not be able to survive. In the 6-day storage period, the pH value is higher, this is due to the breakdown by bacteria to form products such as ammonia which in turn can reduce the acidity value. As noted by Chamidah [45], that during storage the protein breaks down into alkaline compounds, including ammonia. The decrease in acidity value allows the growth of neutrophilic bacteria so that the number of bacterial colonies increases.

Conclusion

Based on the results and discussion, it is concluded that lime juice with different concentrations has a different effect on bacterial growth. The higher the concentration, the less colony growth. The storage period did not have a significant effect on bacterial growth. In the control without lime juice and with lime juice 125 mL/300 g, the number of colonies increased with increasing storage period. In the treatment of lime juice 250 mL/300 g, at the storage period of 2 days and 4 days, the number of bacteria decreased but tended to increase in the 6 day storage period. The study also found no interaction between the concentration of lime juice and the storage period on the number of bacteria in *se'i* beef. In all organoleptic parameters, the bad and very bad categories were found in the 6-day storage period of all treatments with the concentration of lime juice.

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