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Effect of Fermentation Time on Rabbit Meat Jerky Processing

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Abstract

This study aims to develop fermented rabbit meat jerky. *Lactobacillus casei* strain Shirota (Lcs) from commercially available cultured drink was used as a starter culture. Samples of jerky were fermented at 3 h, 5 h, and 7 h. Parameters such as *Lactobacillus* count (CFU/g), pH, % titratable acidity, % moisture content, and water activity (A_w) were investigated. Results revealed that fermented rabbit meat jerky has a significantly lower pH than unfermented. Percent (%) moisture content and A_w of the fermented samples are found to be comparable to other dried fermented meats. However, no significant changes were observed in % titratable acidity among the jerky produced. The results of this study provide baseline data on the microbiological and physicochemical properties of rabbit meat jerky during fermentation.

Keywords: *rabbit meat, Lactobacillus casei, jerky, fermentation*

Introduction

Over the years, fermentation serves as one of the most convenient ways of preserving food. It is a process by which microorganisms such as yeasts or bacteria convert carbohydrates and proteins to alcohol and organic acids under aerobic conditions. Lactic acid fermentation is known to preserve a wide range of foods ranging from vegetables (kimchi, sauerkraut) dairy (cheese, yogurt), and meat (salami and chorizo). Lactic acid bacteria (LAB) such as *Lactobacillus* spp. *leuconostocs* and *lactococci,* can convert sugars into lactic acid. Production of lactic acid during fermentation inhibits the growth of harmful bacteria by lowering the pH while developing the flavour and aroma of the product. Recently, a growing interest occurred in obtaining jerky through fermentation.

Jerky is one of the most popular meat snacks in the world. Its popularity may be due to health benefits and convenience. It can be consumed without preparation and can be easily stored during long trips. Jerky is dried strips of meat. At present, jerky can be produced in different forms: whole-muscle or restructured. Restructured meat products are products that are chopped, ground, or flaked and then form to the desired shape. Binders are added to retain the product's structure (Lornegan and Marple, 2019). Restructuring enables small pieces of meat to be processed.

Among the popular meat that can be used are pork, chicken, and beef. In fermenting meat, an appropriate and suitable concentration of inoculum or starter culture is added to initiate the process followed by spices and or other ingredients. The primary objective of the culture is to induce the growth of concerning microorganism – *Lactobacilli*. Previous studies showed that through this process, the texture, color, and flavor of the jerky improved (Zhao *et al*, 2018). Moreover, the use of beneficial bacteria like LAB to produce a probiotic-rich diet has long been known to prevent and treat digestive orders, hence, considering fermented products as functional foods (Sidira *et al*., 2019)

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Rabbit meat or commonly called *lapan* is one of the major research thrusts of Bulacan Agricultural State College (BASC). Its high protein and low cholesterol, saturated fats, sodium, and caloric count make it the most nutritious meat available to man (Foodstruct, 2020). *Lapan* also contains essential fatty acids making it superior to other types of meat such as pork, chicken, and beef. However, given this information, fewer studies regarding value adding of *lapan* are available. Thus, this study is conducted to develop *lapan* jerky through fermentation.

Methodology

Sample preparation

Whole frozen rabbit meat was purchased from a rabbit farm located in Bulacan. The meat was manually separated from the bone and was ground using an electric meat grinder. Other raw materials used in the study were purchased in a local supermarket in Bulacan.

Product Formulation and Processing

Preliminary trials were conducted to determine the amount of inoculant and length of fermentation. Based on the conducted preliminary trials, the maximum length of fermentation without any noticeable spoilage organoleptic properties is 7 h (Table 2). The amount of spices and additives used in the study were based on the previous studies conducted by Kucerova (2018) with slight modifications. Carrageenan was added as a binding agent and a commercially available probiotic drink (Yakult™) was used as the starter culture. The concentration of the starter culture used in this study was 8.91 log CFU/ml. Table 1 presents the formulation used based on the result of preliminary studies.

Table 1

Product Formulation of Jerky

Ingredients	Unfermented	Fermented
Lapan, g	71.60%	71.60%
Water, mL	24.00%	0.00%
Yakult™, mL	0.00%	24.00%
Spices and additives	4.40%	1.00%
Total	100.00%	100.00%

The ingredients were mixed and fermented at 35 $\pm 2^{\circ}$C. The length of fermentations was shown in table 2. The mixtures were transferred in a piping bag. Samples were piped out at a size of 4.26 mm x 2.5mm x 3 mm in a tray lined with wax paper and dried at 75°C for 2 hours, then it was further dried at 90 °C for 30 mins.

Table 2

Treatments used in the Study

Treatments	Length of Fermentation
A	Control (without microbial starter culture)
B	Inoculated with Yakult™ and fermented for 3 hours
C	Inoculated with Yakult™ and fermented at 35$\pm$2°C for 5h
D	Inoculated with Yakult™ and fermented at 35$\pm$2°C for 7h

Microbiological analysis

Lactobacillus Count (CFU/g)

Lactobacillus count (CFU/g) of the jerky samples was determined using pour plate method. A 10-g sample was homogenized and serially diluted up to 10^{-7} using sterile phosphate buffer solution. 1-ml sample of the last two dilutions was dispensed in each Petri plate followed by the addition of 20-ml De Man, Rogosa, and Sharpe (MRS; HiMedia) agar. The plates were allowed to solidify and were incubated at 35$\pm$2°C for 72 hours.

Physicochemical analysis

pH Values. The pH was determined in accordance with the method of Zhao (2015) with slight modification. The pH of the jerky samples was determined after the fermentation and drying process. A 5-gram sample was cut and blended with 45 ml distilled water for 5 minutes and then filtered. The pH of the filtrate was then obtained using a handheld pH meter (Milwaukee pH 600).

Titratable Acidity (%). The percent (%) titratable acidity after fermentation and drying was determined. A 5-g sample was mixed with 45 ml distilled water. To the mixture, a 1.0-ml phenolphthalein indicator was added. The sample mixture was titrated against a standardized 0.1 N NaOH. The endpoint was obtained when a faint pink color persist for 10 seconds. The volume of NaOH used to reach the endpoint was considered for the computation of the % titratable acidity. The percent titratable acidity was calculated as follows.

$$\%TA=\frac{\text{Vol of NaOH x Normality of NaOH x}\frac{90}{1000}}{\text{sample weigh in g}} \text{X } 100\ \%$$

Moisture Content (%). Moisture content of samples was determined using oven-drying method (AOAC, 1991). Samples were dried at 105°C until constant weight was achieved. Moisture content was calculated as follow:

$$\%MC=\frac{\text{Weight before drying-weight after drying}}{\text{weight before drying}} \text{X } 100\ \%$$

Water activity. Water activity of the samples was measured using water activity analyzer (Biobase Model No. BWA - 3A). Samples were chopped and placed in a plastic cup container with cover inside the water activity sensor.

Statistical analysis and interpretation

All analyses were done in triplicate. Data obtained were analyzed using Analysis of Variance (ANOVA) and Duncan's multiple range test was used to compare means at a 5% significance level.

Results and Discussion

Lactobacillus Count

Rabbit meat, like any other meat, is proteinaceous. Next to water, the major component is protein (Philippine Food Composition Table, 2022). Upon addition of LAB, this protein along with other constituents like carbohydrates and lipids are slowly metabolized and consumed by microorganisms. This allows them to multiply and grow in numbers and consequently produced organic acids (primarily in the form of lactic acid). The starter culture used in fermenting *lapan* jerky is a commercially available probiotic drink (Yakult™). The solution contains *Lactobacillus casei strain Shirota* (LcS). The initial lactobacillus count of the culture solution was 8.91 log CFU/ml. During fermentation, the amount of LAB is expected to increase up to 8 logs CFU/g and should be maintained at 6 to 7 log CFU/g throughout the lifespan of the product. As shown in Table 3, microbial count (log CFU/g) remains within the range of 8.32 to 8.43 log CFU/g.

Fermentation beyond 7 h renders the *lapan* jerky undesirable, as it starts to produce an unwanted odor. This disadvantageous development can be attributed to the presence of spoilage-causing microorganisms in the raw rabbit meat. The microflora of rabbit meat is known to be very complex. Microorganisms like *Pseudomonas*, *Enterobacteriaceae*, coliforms, LAB, yeasts, and molds are present in significant amounts in rabbit carcass (Rodriguez-Calleja et. al., 2004). The presence of these microorganisms may inhibit the growth of LAB by competing with the available nutrients present in the meat.

Table 3

Microbial count (log CFU/g) of lapan jerky at various lengths of fermentation

Length of fermentation (h)	Microbial count (log CFU/g)
3	8.32
5	8.66
7	8.43

LAB proliferate best at 35±2 °C. Given the appropriate temperature, even in the presence of minimal nutrients, these microorganisms can proliferate. Since *lapan* is abundant in protein (Table 4), LAB tends to use this instead as their source of energy, which then results in proteolysis and lipolysis (for the lipids present). Unlike carbohydrates, metabolic products of *Lactobacillus casei* from proteins and lipids are related to the production of flavors and aroma (Adams, 2010), hence, prolonged fermentation may result in off-flavor and odors as observed in the preliminary study of this research.

Bekasam, a fermented product from Indonesia, which is usually made from fish is now made using rabbit meat. Wulandari et al., (2020) evaluated the probiotic lactic acid bacteria diversity of rabbit meat bekasam-fermented meat. Results showed that the highest group of microorganisms present is

LAB (8.67 log CFU/g) followed by yeast (8.58 log CFU/g). This shows that the microbiological character of fermented *lapan* jerky, is somewhat similar to the same type of products, such as bekasam.

Table 4

Proximate composition of rabbit meat

Proximates (g)	Amount per 100 g
Water	78.4
Protein	18.2
Total Fat	2.4
Carbohydrate, total	0.0
Ash	0.1

Reference: Philippine Food Composition Table, DOST-FNRI

In preserving foods through fermentation, the rate of fermentation is one of the crucial parameters. Jerky can be processed either with slabs of meat or ground meat. Due to the carcass composition of rabbit (Hernández and Blasco, 1996), the researchers opted to use ground meat. In fact, the increased surface area allows for a faster rate of fermentation, as more water becomes available for biochemical reactions of microorganisms. Moreover, as the fermentation rate increases in favorable temperature (35 ± 2 ◦C), it should not be overlooked that some spoilage and pathogenic microorganisms such as *Enterobacteriaceae*, may grow as well with LAB, thus may increase the risk of foodborne illness (Jay, 2000). For this reason, it is precisely critical to choose the appropriate fermentation hours.

pH Change After Fermentation and After Drying

Table 5

Mean Values for pH of Fermented Jerky after Fermentation and after Drying

Treatments	pH	
	After Fermentation	After Drying
A	6.31 ± 0.16^{a}	6.39 ± 0.16^{a}
B	5.85 ± 0.05^{b}	6.21 ± 0.13^{b}
C	5.73 ± 0.08^{b}	6.07 ± 0.12^{b}
D	5.73 ± 0.05^{b}	6.05 ± 0.03^{b}

Legend: Mean ± standard deviation.
Means with different superscripts within the same column are significantly different at *p* (<0.05).
A: Control (without microbial starter culture).
B: Inoculated with Yakult and fermented at 35 ± 2^{0}C for 3h.
C: Inoculated with Yakult and fermented at 35 ± 2^{0}C for 5h.
D: Inoculated with Yakult and fermented at 35 ± 2^{0}C for 7h.

A pH measures the acidity or basicity of a solution. Treatment A has a significantly higher pH than the other treatments. Treatment A has no starter culture and possibly did not undergo fermentation. Thus, it is expected that it would have a higher pH. On the other hand, no significant differences were observed among jerky with Yakult™ fermented at 3h, 5h, and 7h. During fermentation, the *Lactobacillus casei* present in Yakult produces lactic acid and other organic acids that in turn reduce the pH of the jerky. However for this LAB to cause a significant amount of acids and to significantly lower the pH, sufficient time may be needed. As shown in this study, fermentation of 7h was insufficient to cause a significant reduction in pH. The same was observed in the study of Fadlillah et al (2020), wherein fermentation of rabbit meat using *L. plantarum* for 12 to 18h did not result in a significant reduction in its pH.

According to Zhao et al (2015), a lower pH in meat promotes the reduction of sodium nitrite and decreased the amount of residual nitrite, thereby reducing carcinogens- nitrosamines produced by nitrite and secondary amines. After drying, an increase in pH was observed, but still, the unfermented jerky (treatment A) is significantly higher than the fermented jerky (Treatment B, C, and D). The slight increase in pH after drying may be attributed to the loss of moisture and other volatile organic acids, which then results in a more meat-rich product. Determining pH is one of the key determinants of LAB activity. As LAB grows in number, the production of lactic acid as well as other acids increases, thus, reducing the pH of product. This decrease contributes directly to microbial safety as some spoilage and pathogenic microorganisms have no ability to tolerate lower pH (Adams and Moss, 2008). The distinct sour-like characteristics of fermented products are also attributed to this parameter.

Titratable Acidity (TA) after fermentation and after drying

The percent (%) titratable acidity of the samples after fermentation ranged from 0.249 to 0.262% expressed as lactic acid (Table 6). A slight increase in the % TA was observed after drying. This is due to the removal of moisture during drying thereby resulting in a more concentrated product. Naturally, meat contains lactic acid. After the animal has been slaughtered, the glycogen in the meat is converted into lactic acid (FAO, 2020). During fermentation, additional lactic acid is produced. Yakult™ contains *Lactobacillus casei*, a gram-positive lactic acid bacterium (Lorenzo, 2018). The lactic acid bacteria (LAB) produce lactic acid as their major fermentation product. However, despite this, no significant increase was observed in all fermented products.

Table 6

Mean Values for %TA of Fermented Jerky after Fermentation and after Drying

Treatments	% Titratable Acidity	
	After Fermentation	After Drying
A	0.249±0.012	0.320±0.012
B	0.253±0.009	0.343±0.014
C	0.257±0.022	0.293±0.053
D	0.262±0.045	0.369±0.034

Legend: Mean ± standard deviation.
Means with different superscripts within the same column are significantly different at p (<0.05).
A: Control (without microbial starter culture).
B: Inoculated with Yakult and fermented at 35±2°C for 3h
C: Inoculated with Yakult and fermented at 35±2°C for 5h.
D: Inoculated with Yakult and fermented at 35±2°C for 7h.

During fermentation, microorganisms, in general, utilize the available nutrients present, such as carbohydrates, proteins, and fat as their source of energy. One of the byproducts of their metabolism is the production of organic acids, which cause fermented products to have lower pH and a distinct sour taste. In the case of LAB, the major metabolic end-product is lactic acid, and this is produced when carbohydrate sources were metabolized (Adams, 2010). Since rabbit meat has no carbohydrates (Table

4) and the supplemented carbohydrate source is minimal, it is not enough to support the production of lactic acid.

Water Activity and Moisture content

Water activity (*A_w*) is a measure of the availability of water for biological functions and relates to water present in a food in a free form (Ray, 2005). Microorganisms utilized this water to grow and multiply. Thus, determining the *A_w* is crucial to food stability and food safety. The *A_w* of the jerky after drying ranges from 0.84 to 0.87 (Table 7). Minimizing the *A_w* is critical in inhibiting the growth of pathogens. An *A_w* of 0.85 or less is recommended in ready-to-eat products like jerky in order to control the growth of harmful microorganisms (FSIS, 2012). The values reflected in this paper are somewhat comparable but still, additional interventions should be done to achieve a much lower *A_w*, and in turn, produce a safer product.

Table 7

Mean Values of A_w and % Moisture content of Fermented Jerky after drying

Treatments	A_w	% Moisture content
A	0.874$\pm$0.009	38.51$\pm$5.51
B	0.854$\pm$0.024	30.66$\pm$2.31
C	0.860$\pm$0.009	33.40$\pm$1.20
D	0.844$\pm$0.021	28.31$\pm$0.36

Legend: Means with different superscripts within the same column are significantly different at p (<0.05).
A: Control (without microbial starter culture).
B: inoculated with Yakult and fermented at 35$\pm$2^{o}C for 3h
C: Inoculated with Yakult and fermented at 35$\pm$2^{o}C for 5h.
D: Inoculated with Yakult and fermented at 35$\pm$2^{o}C for 7h.

In this study, the moisture content of the *lapan* jerky ranged from 28.31% to 38.51%. Drying has been traditionally used to preserve meats. The moisture content (MC) affects the stability, safety, and sensory properties of foods. The values obtained in this study are comparable to other fermented meats like dried salami and pepperoni where moisture content ranges from 20-45% and *A_w* of 0.85-0.86 (Ockerman and Basu, 2014). Considering the *A_w* and moisture content of all treatments, the jerky produced can be classified as intermediate moisture foods (IMF). These are foods that have a moisture content of 10-50% and *A_w* 0.65-0.90 (Batt & Tortorello, 2014). Bacteria cannot grow on IMF due to its low *A_w*, however, yeasts and molds may grow (Ray, 2005).

Conclusion and Recommendations

The study shows the effect of fermentation on the microbiological and physicochemical properties of rabbit meat jerky. The resulting product after fermentation has a high moisture content, pH and Aw, and low % TA which may shorten its shelf life. Hence, intervention in the process and formulation is suggested. To increase the production of lactic acid as indicated by %TA and pH, it may be necessary to provide additional sources of carbohydrates in the formulation as substrates for *L. casei* and prolong the fermentation hours. Rabbit meat contains complex microflora thus, pretreatment of the raw rabbit meat prior to fermentation to eliminate undesirable microorganisms must be taken into consideration. In addition, other byproducts during fermentation should also be monitored such as free amino acids.

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