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Effect of adjunct inoculation of *Lactobacillus plantarum* BS and *Pediococcus acidilactici* 3G3 on the microbiological, physicochemical and sensory properties of fermented carabeef (*pindang damulag*)

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Abstract

Lactobacillus plantarum BS and *Pediococcus acidilactici* 3G3 were inoculated singly or jointly on carabeef (*pindang damulag*) and allowed to ferment at 10 and 32°C for five days, in comparison with uninoculated naturally fermenting samples. Greater pH reduction and increased lactic acid production was observed in samples inoculated with the lactic acid bacteria (LAB) starter cultures and those fermented at 32°C. Fermentation increased the monounsaturated fatty acid content of carabeef which may have good health benefits. The total plate count, coliform count, yeasts and molds count generally decreased after fermentation for five days which were more pronounced in LAB-inoculated samples. It was also observed that acid-forming bacteria were the predominant microflora in all fermented samples. Sensory evaluation revealed that the addition of starter cultures significantly improved flavor and general acceptability of fermented carabeef. The most acceptable was the meat inoculated with a combination of *L. plantarum* BS and *P. acidilactici* 3G3. Samples fermented at lower temperature (10°C) had significantly higher overall acceptability scores than those fermented at 32°C. The study demonstrated the potential of LAB starter cultures in improving safety, nutritional and sensory properties of *pindang damulag*.

1. Introduction

Meat plays a huge role in the human diet, being the major source of animal protein in the world. Among the different types of meat, red meats including beef and carabeef (buffalo meat) are often preferred by consumers. Red meats have been discovered to be superior over white meat in their fatty acid profile (Wyness *et al*., 2011). Carabeef is not being consumed by people as much as beef, but has been becoming popular worldwide because of its lower price and inherent properties believed to be superior over beef including lower intramuscular fat, cholesterol and calories as well as higher units of essential amino acids, biological value and iron content (Aziz *et al*., 2014).

In the Philippines, one traditional product is the naturally fermented carabeef cuts, locally known as *pindang damulag*, which is popular in the northern part of the country, specifically in Central Luzon, and is thought to have originated in Pampanga province. The processing of *pindang damulag* has often been done in households at small to medium scale thus there has not been any established or standardized processing method. *Pindang damulag* is among those traditional foods that the country prizes but is little exploited in terms of mass and commercial processing as there have been little efforts undertaken to commercialize the product.

Nowadays, the use of selected starter cultures or inocula for fermentation is becoming increasingly popular and necessary. Fermentative microorganisms help facilitate optimum fermentation process. Addition of starter culture is known to reduce the fermentation time, extend the shelf life and enhance the organoleptic characteristics of the final product (El Adab *et al*., 2014). Most of the starter cultures used in meat fermentation involve the use of a single strain or a combination of

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strains of lactic acid bacteria or LAB (Ammor and Mayo, 2007). This study determined the effects of LAB starter cultures on the microbiological stability, physicochemical properties sensory characteristics, and free fatty acid content of *pindang damulag.*

2. Materials and methods

2.1. Preparation of inoculum

Lactobacillus plantarum BS and *Pediococcus acidilactici* 3G3, both previously isolated from fermented rice-shrimp mixture and fermented pork, respectively (Banaay *et al*., 2004; Ahmadian-Moghadam and Elegado, 2017), were grown in de Mann, Rogosa and Sharpe (MRS) medium (Pronadisa, Spain). All prepared media were sterilized by autoclaving for 15 mins at 15 psi. Glycerol stocks of the cultures (0.5 mL) in Eppendorf tubes were obtained from the biofreezer and revived by adding to 5 mL of MRS broth in screw-capped test tubes and incubating at 37ºC for overnight. The 5 mL cultures were transferred in 500 mL MRS broth and cultured at 37ºC for overnight. After which, 100 g of oven-sterilized maltodextrin was added to the culture broth and subjected to low-temperature spray drying (YK-100, True Ten Industrial Co., Taiwan) using an inlet temperature of 45ºC and outlet temperature of 40ºC. The spray dried powder inocula were collected and kept in sterile autoclavable polypropylene plastic bags at 4ºC until use.

2.2. Preparation of fermented carabeef

The curing formulation for every kilogram of carabeef cuts included 150 g sugar (15%), 20 g salt (2%), 2.5 g prague powder (0.25%), 3 g potassium phosphate (0.30%) and 100 mL water (10%) (Martin, 2001).

Carabeef cuts were tempered overnight in a cold storage facility (4°C) prior to cutting into uniform thin slices. Curing ingredients were thoroughly mixed and massaged onto sliced carabeef for about 5 mins until all the ingredients are fully incorporated. Four batches of meat mixtures were prepared corresponding to 4 treatments (6 kg for each treatment): T_1: control without inoculation, T_2: inoculated with spray-dried *L. plantarum* BS (1g/1kg), T_3: inoculated with spray-dried *P. acidilactici* 3G3 (1g/1kg) and T_4: inoculated with a combination of *L. plantarum* BS and *P. acidilactici* 3G3 (1:1 at 1g/1kg). Initial counts for *L. plantarum* BS and *P. acidilactici* 3G3 starter culture powders are 6.30 x 10^6 and 6.47 x 10^6 CFU/g carabeef, respectively.

Meat mixtures were placed in food grade polypropylene bags at 500 g per bag and sealed. Meats were fermented for five (5) days at two different temperatures (10 and 32°C), with each treatment-temperature combination done in triplicate. Initial microbial and physicochemical analyses were done prior to fermentation. At the end of the fermentation period, samples were taken for microbiological, physicochemical and sensory analyses, with each analysis carried out in triplicate.

2.3. Microbiological enumeration

Meat samples (25 g) from each batch were homogenized with 225 mL of sterile 0.85% NaCl solution and decimal dilutions of 10^{-1} to 10^{-8} were prepared. Total viable counts were determined on standard plate count agar after incubation at 30°C for 48 hrs. Acid-forming bacteria which include mesophilic LAB were enumerated on MRS agar with 1% $CaCO_3$ after 48 hrs of incubation at 30°C. On the other hand, yeasts and molds were enumerated on Potato Dextrose Agar (PDA) with 10% tartaric acid at 37°C after 2-3 days. Presence of coliforms was determined using Violet Red Bile Agar (VRBA) upon incubation at 37°C for 24 hrs.

2.4. Physicochemical analysis

2.4.1. pH

Meat samples were tested for pH using a digital pH meter. The electrode of pH meter was calibrated using two buffer solutions of known pH, 4 and 7. A total of 10 g of finely ground sample was taken and blended in 50 mL of distilled water in a test tube and mixed in a vortex mixer. The extract was filtered through Whatman No.1 filter paper and the pH of the sample was recorded.

2.4.2. Titratable acidity (expressed as % lactic acid)

Lactic acid content of samples before and after fermentation was determined following the methods of AOAC (2010). A total of 10 g of meat samples were homogenized with 50 mL distilled water and filtered using Whatman No.1 filter paper. An aliquot of 5ml was placed in a flask, added with 20 mL distilled water and titrated against standardized 0.01 N NaOH, with phenolphthalein as indicator. Percent lactic acid was computed using the formula:

$$\%\ Lactic\ acid = \frac{Vol.of\ titrant\ used\ (mL) \times N\ of\ titrant \times Eq.wt.of\ acid\ (0.090\ for\ lactic\ acid)}{\left(\frac{Wt.of\ sample\ (g)}{Vol.of\ water\ added + Wt.of\ sample}\right) \times Vol.of\ aliquot} \times 100$$

2.4.3. Total soluble solids (TSS)

Total soluble solids were determined in duplicate using Abbe 60 Refractometer and the results were expressed as degree Brix (°Brix). A drop of the sample mixture is placed on graduated prism of the meter and closed; the reading was taken by viewing through the refractometer and recording the values on the graduated slide. Three readings were taken and the mean was recorded.

2.4.4. Proximate analysis

The meat sample that obtained the most acceptable sensory score was analyzed at the Animal Nutrition Analytical Service Laboratory, Animal and Dairy Science Cluster, UPLB for proximate composition. Analyses for moisture, ash, crude protein and crude fat were carried out in triplicate, following AOAC protocols (2000).

2.4.5. Free fatty acid content determination

The meat sample treated with adjunct inoculants that got the highest acceptability score in the sensory evaluation was analyzed for fatty acid profile. Samples were sent to the Department of Science and Technology – Industrial Technology Development Institute (DOST-ITDI) Standards and Testing Division for fatty acid profiling. Fat extraction from meat was done following AOAC Official Method 991.36 (AOAC, 2012a). Fatty acid profiling was done using gas chromatography following AOAC Official Method 963.22 (AOAC, 2012b). The analysis was carried out in three trials for each sample with duplicate samples per treatment.

2.5. Sensory evaluation

Cooked *pindang damulag* were subjected to sensory evaluation. The samples were fried in vegetable oil (low heat, 12 mins) prior to evaluation. Sensory evaluation was done using the 9-point hedonic scale with students and staff of the Institute of Food Science and Technology, College of Agriculture and Food Sciences, University of the Philippines Los Baños as panel members (24-member panel). Sensory qualities evaluated include color, flavor, tenderness, juiciness, and general acceptability. Numerical values ranging from 1 to 5 were given as the descriptive scores as follows: Color: 5-Dark Brown, 4-Brown, 3-Reddish Brown, 2-Dark Red, 1-Red; Flavor: 5-Very sour, 4-More perceptible sour than sweet, 3-Right combination of sweet and sour, 2-More perceptible sweet than sour, 1-Bland; Off-Flavor: 5-Very Strong, 4-Strong, 3-Moderately strong, 2-Slightly perceptible, 1-None; Tenderness, 5-Very tender, 4-Tender, 3-Neither tender nor tough, 2-Tough, 1-Very tough and Juiciness: 5-Very juicy, 4-Juicy, 3-Neither juicy nor dry, 2-Dry, 1-Very dry. Acceptability scores are described as follows: 9-like extremely; 8-like very much; 7-like moderately; 6-like slightly; 5-neither like nor dislike; 4-dislike slightly; 3-dislike moderately; 2-dislike very much; and 1-dislike extremely.

2.6. Statistical analysis

Data on the microbial count, physicochemical and sensory properties of the variously treated carabeef were statistically analyzed using analysis of variance (ANOVA) of SPSS 17.0 software (SPSS, Inc., Chicago, IL). Tukey's HSD test was employed to further determine which groups in the samples differ significantly.

3. Results and discussion

3.1. Microbiological properties of fermented carabeef

Figure 1 shows the evolution of total viable counts, lactic acid bacteria (LAB), yeasts and molds, and coliforms before and after fermentation as affected by starter culture and fermentation temperature. Results showed that the addition of selected starter cultures significantly affected ($p>0.05$) the quantitative evolution of different microbial groups, whereas the growth of microorganisms has been significantly affected ($p<0.05$) by the fermentation temperature. The numbers of total viable counts significantly decreased after fermentation, with population found to be higher in meats inoculated with starters compared with uninoculated samples, basically due to the prior inoculation of starter cultures. The decrease in the viable counts after fermentation can be attributed to the inhibitory effect of pH reduction to microbial growth and survival. It might also be due to inhibitory effects of curing salt (prague powder) on the growth and metabolic activity of microorganisms as well as moisture loss during fermentation (Gençcelep *et al*., 2007).

LAB had been one of the dominant microflora in both control and starter inoculated samples. Starter cultures, fermentation temperature and the interaction of both factors significantly affected the survival of LAB in the meat samples ($p<0.05$). As expected, treatments inoculated with starter cultures have significantly higher LAB counts than uninoculated samples after fermentation. This is because the survival of LAB during fermentation is also dependent on the initial microbial load. Higher LAB counts were also observed in samples fermented at 10°C. It was reported that some LAB strains are well adapted to low temperatures (Ammor and Mayo, 2007). The decrease in LAB counts may be due to the exhaustion of the sugar as they are converted to lactic acid, thereby limiting energy sources for microorganisms (Fernandez-Lopez *et al.,* 2008). This was more evident at 32°C due to faster metabolism. The higher pH reduction may also pose some stress to the microorganisms, thereby affecting their growth and survival.

A significant decrease ($p<0.05$) was observed in the viable counts of yeasts and molds after fermentation while LAB dominated the microbial flora of carabeef samples. More importantly, no molds were detected in the samples after fermentation. The final counts refer

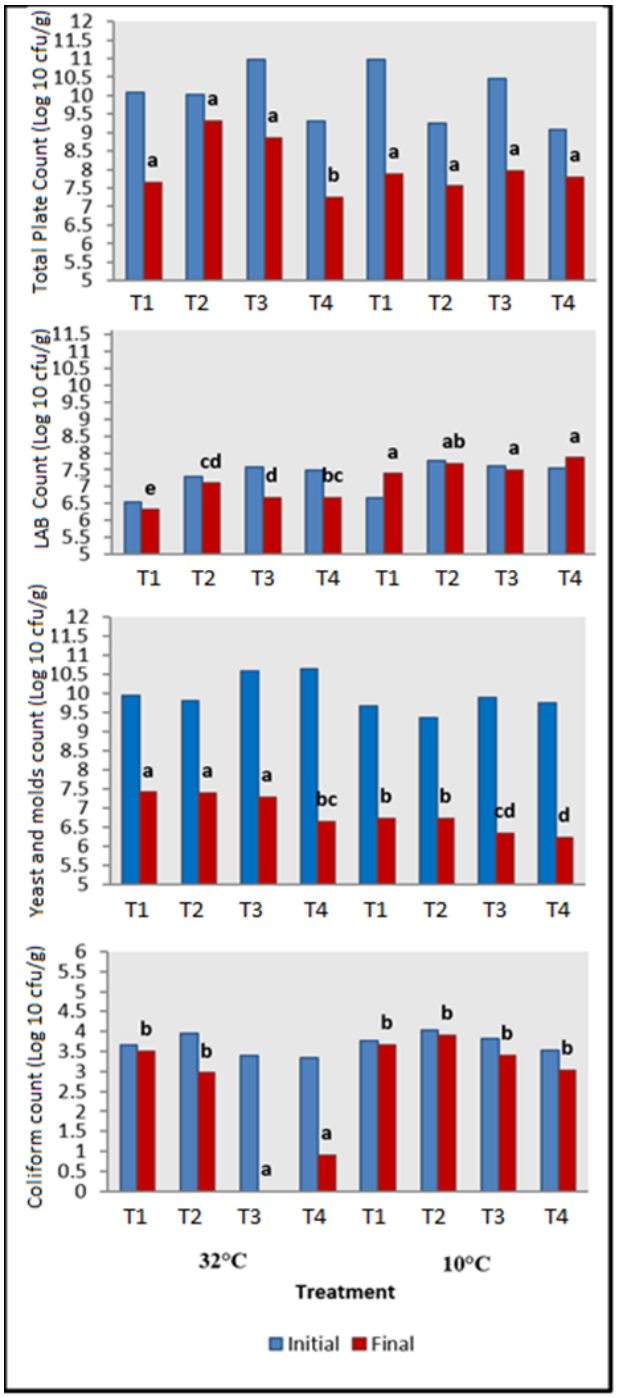

Figure 1. Mean microbial population counts (Log10 CFU/g) of carabeef samples before and after fermentation. Means of the same parameter having the same letter are not significantly different at 5% level of significance according to Tukey's HSD. (T1=No Starter culture; T2=*L. plantarum* BS; T3=*P. acidilactici* 3G3; T4=Combination of *L. plantarum* BS and *P. acidilactici* 3G3)

strictly to yeast counts. This can be due to the reduction of pH in the carabeef samples during fermentation that ranged from 3.67 to 4.23, low enough to inhibit mold growth (Betts, 2015). Furthermore, molds are aerobic organisms and since the fermentation set-up was geared towards anaerobic to microaerophilic conditions, mold growth was inhibited. Several yeasts have higher adaptability to adverse conditions such as low pH, low water activity, and even in the presence of some common chemical preservatives (Betts, 2015), explaining why yeasts were still detected at the end of fermentation of carabeef samples.

Coliform counts decreased at the end of fermentation for both inoculated and uninoculated treatments. Starter cultures had a significant effect ($p<0.05$) on the reduction of coliform counts of samples after fermentation. Final coliform counts are significantly lower in meat samples inoculated with starter cultures, specifically *P. acidilactici* (Treatment 3) which totally eliminated the coliform. This demonstrates the antagonistic property of the starter cultures against coliforms due to pH decrease and LAB production of other antimicrobial compounds such as peroxidases and diacetyls (Papamanoli *et al*., 2003; Leroy *et al*., 2006; Casaburi *et al*., 2008), which were more significantly pronounced ($p<0.05$) at higher temperature (32°C) than at (10°C) (Zhang *et al.*, 2010).

3.2. Physicochemical properties of fermented carabeef

Table 1 shows the final pH, TSS and lactic acid content of 7-days fermented meat samples as affected by starter culture and fermentation temperature. The pH of the meat samples decreased from average values of 5.3-5.6 to 3.67-4.23. There was a significantly higher drop in the pH for samples inoculated with starter cultures and incubated at 32°C. The highest pH drop was observed in the meat inoculated with *L. plantarum* BS. The decrease in pH is presumably caused by an accumulation of organic acids, mainly lactic, as a result of carbohydrate breakdown during fermentation (Zaho *et al*., 2011).

The addition of starter cultures and temperature differences had significant effect ($p<0.05$) on the pH drop of the meat samples. This result is in accordance with the findings of El Adab and co-workers (2014) with fermented sausages. Carabeef incubated at 32°C have significantly lower final pH values than those fermented at chilling temperature (10°C) due to the increased microbial activity and the increased production of organic acids. The use of starter cultures had significantly higher increase in %TA (2.04%) in samples inoculated with *L. plantarum* BS and fermented at 32°C. Although not significantly different, the samples inoculated with *P. acidilactici* 3G3 and a combination of the two LAB strains also had higher increase in lactic acid content after fermentation (1.75% and 1.65%, respectively) than those that were not inoculated with starter culture (1.46%). Between the starter cultures used, *L. plantarum* BS has been more efficient in converting fermentable sugars in the meat samples to lactic acid. This agrees with the results of a study by Wiriyacharee (1990), in which the addition of *L. plantarum* accelerated very distinctly the decrease in the pH of *nham* (Thai

Table 1. Changes in the pH of fermented carabeef as affected by starter culture and fermentation temperature.

Fermentation Temperature	Treatment	Physicochemical Properties		
		pH Drop	Increase in L.A. (%)	TSS Drop (°Brix)
32°C	T1	1.48±0.14^{b}	1.46±0.18^{b}	7.53±1.62^{c}
	T2	1.75±0.20^{a}	2.04±0.06^{a}	15.62±0.19^{a}
	T3	1.74±0.12^{ab}	1.75±0.10^{b}	10.47±0.20^{cb}
	T4	1.63±0.03^{ab}	1.65±0.03^{b}	13.02±1.98^{b}
10°C	T1	1.14±0.05^{c}	1.12±0.16^{c}	9.03±0.38^{b}
	T2	1.24±0.10^{bc}	0.83±0.05^{d}	10.77±0.39^{cb}
	T3	1.13±0.03^{c}	0.67±0.06^{d}	7.11±0.30^{c}
	T4	1.11±0.02^{c}	0.82±0.07^{d}	7.68±0.57^{c}

N=3; Means with a common letter superscript within a column are not significantly different at 5% level of significance based on Tukey's HSD. (T1= No Starter culture; T2= *L. plantarum* BS; T3= *P. acidilactici* 3G3; T4= Combination of *L. plantarum* BS and *P. acidilactici* 3G3).

fermented lean pork). Lactic acid production is affected by factors including type and amount of carbohydrate (sugars) in the substrate available for fermentation and microbial activity (Cocolin and Rantsiou, 2012).

The initial TSS of the meat samples were more or less made uniform through the addition of the same amount of washed sugar to the meat mixture. A decrease in the TSS was observed for all meat samples after fermentation. This decrease in TSS is likely a result of the action of fermentative microbes on the carbohydrate content of the meat system, utilizing them as the substrate for lactic acid fermentation. It was observed that there was a significantly higher reduction of TSS content in samples inoculated with starter cultures, particularly with *L. plantarum* BS. This decrease in TSS is also manifested in the increase in lactic acid content and decrease in pH of the fermented meat samples. The highest decrease in TSS was recorded at 15.62°Brix, in the meat inoculated with *L. plantarum* BS. This treatment also recorded the highest pH drop and lactic acid content increase. This high decrease in TSS after fermentation may be due to higher efficiency of *L. plantarum* BS to convert sugars into lactic acid. Results also showed that there was significantly lower TSS drop in the samples fermented at 10°C compared to those fermented at 32°C due to lower LAB activity.

3.3. Sensory properties of fermented carabeef

Besides contributing to human health, probiotic fermented meats need to be of sufficient commercial value. Therefore, the sensory quality of fermented meats remains to be a primary requirement, as for any other fermented products. The sensory characteristics including color, sourness, off-flavor, tenderness, juiciness and overall acceptability of fermented carabeef were evaluated. The mean scores given by panelists for each treatment are presented in Table 2.

3.3.1. Color

The perceived color of the samples was not significantly affected by starter culture and fermentation temperature ($p<0.05$). Panelists described cooked meat samples as brown to reddish brown. In cured meat and meat products, myoglobin can exist in different forms. Because cooking denatures globin and converts residual deoxymyoglobin and oxymyoglobin to metmyoglobin, NO-Myoglobin (pink) and metmyoglobin (brown) would be the main pigments present in cooked cured meats. Nitrosohemachrome is a denatured, stable form of NO-Mb in cooked, cured meats (Martin, 2001). This renders the observed color for the cooked samples as somewhere between reddish-brown to brown.

3.3.2. Flavor

A characteristic flavor of fermented products is sourness due to lactic acid. In the study, the perceived sourness was significantly affected by starter culture and fermentation temperature ($p<0.05$). In terms of starter culture, Treatment 2 (inoculated with *L. plantarum* BS) fermented at 32°C was perceived to be more sour than sweet due to the lower pH and higher lactic acid content. Other treatments were observed to have the right combination of sweetness and sourness, especially those fermented at 10°C which was significantly different ($p<0.05$) than those fermented at 32°C, regardless of starter culture used. Lactic acid production was higher. Furthermore, the perceived flavor of fermented samples was significantly different ($p<0.05$) to the non-fermented samples. Non-fermented samples were observed to be sweeter as the added sugar in the meat mixture was not fermented and there was no production of lactic acid.

3.3.3. Off-flavor

Fermented samples exhibited an off-flavor that was described by the panelists as slightly perceptible to moderately strong. The intensity of off-flavor for fermented samples was not significantly affected

Table 2. Sensory properties of fermented carabeef as affected by starter culture and fermentation temperature.

Treatment	Sensory Properties				
	Color	Flavour	Off-flavor	Tenderness	Juiceness
Control	3.21±1.21[a]	2.16±0.33[c]	1.76±0.93[b]	2.07±0.56[b]	4.57±0.61[b]
Fermented at 32°C					
T1	3.23±1.11[a]	3.46±0.83[ab]	2.91±1.15[a]	3.68±0.84[a]	3.27±0.82[a]
T2	2.73±1.03[a]	3.95±0.78[a]	2.73±1.24[a]	3.77±1.02[a]	3.23±1.02[a]
T3	3.23±0.97[a]	3.55±0.74[ab]	2.55±1.22[a]	3.95±0.65[a]	3.36±0.73[a]
T4	3.18±0.91[a]	3.45±0.96[ab]	2.64±1.09[a]	4.05±0.65[a]	3.41±0.91[a]
Fermented at 10°C					
T1	3.09±0.81[a]	3.18±0.96[ab]	2.50±1.01[a]	3.82±0.85[a]	3.45±1.10[a]
T2	3.18±0.33[a]	2.68±0.95[b]	2.64±1.09[a]	3.82±0.73[a]	3.45±1.14[a]
T3	3.09±1.19[a]	3.36±1.33[ab]	2.23±1.02[a]	3.68±0.65[a]	3.36±0.79[a]
T4	3.32±0.94[a]	3.14±1.04[b]	2.50±1.01[a]	3.50±1.18[a]	3.45±1.01[a]

N=24; Means with a common letter superscript within a column are not significantly different at 5% level of significance based on Tukey's HSD. (T1= No Starter culture; T2= *L. plantarum* BS; T3= *P. acidilactici* 3G3; T4= Combination of *L. plantarum* BS and *P. acidilactici* 3G3).

($p>0.05$) by starter culture and fermentation temperature. However, they significantly differ ($p<0.05$) from the off-flavor intensity observed for non-fermented (control) meat samples. The development of off-flavor is an effect of the fermentation process as well as the cooking process. Fermentation can lead to protein degradation as a result of chemical and enzymatic processes which contributes to the development of off-flavors in meat. Cooking (thermal processing) also causes meat products to develop off flavors, principally because of a cascade of oxidation processes (McKeith *et al*., 1985).

3.3.4. Tenderness

Cooked fermented samples were observed to be significantly tender ($p<0.05$) than the control (non-fermented) samples, which was perceived to be tough. Generally, all fermented samples were observed to have improved tenderness, with no significant difference ($p<0.05$), regardless of starter culture and fermentation temperature employed. This indicates that fermentation process has an effect on tenderness of carabeef muscles, giving the product more desirable textural properties. This is attributed to pH reduction due to acid production during fermentation. Previous studies showed that the tenderness of beef muscle is improved at acidic conditions below the typical pH of postmortem beef muscle (pH 5.2-5.5). The results are in agreement with the results of a study by Li and Zan (2012), in which the addition of lactic acid improved tenderness of beef muscle. In some marination processes, acid injection has been utilized to improve the tenderness of beef muscle.

3.3.5. Juiciness

Juiciness descriptive scores for fermented meat samples were not significantly affected ($p>0.05$) by starter culture and fermentation temperature. Panelists observed the juiciness of meat samples as a range from neither juicy nor tough to juicy. However, juiciness was observed to be significantly ($p<0.05$) different between the fermented samples and the control. The non-fermented samples were perceived to be juicier than the fermented ones. Juiciness is said to be affected by moisture retention of meat samples before and after cooking. Jaroslav (2002) found that moisture retention and drip losses in meat are influenced by factors including size of meat cut, postmortem, storage temperature and the most important factor is the pH value of the meat. As pH of the meat decreases, its water retention decreases (water holding capacity increases). Since the pH of the samples decreased during fermentation, it is expected that their water retention decreases and drip losses increases. This explains the difference in the juiciness of the non-fermented (control) and fermented meat samples.

3.3.6. Acceptability scores

The mean acceptability scores given by 24 consumer panelists for each sensory attribute as well as the general acceptability of the meat samples are presented in Table 3. The general acceptability of the fermented samples was significantly affected by starter culture and fermentation temperature ($p<0.05$). Samples fermented at lower temperature (10°C) generally had significantly higher overall acceptability scores than those fermented at 32°C. Acceptability scores for color, off-flavor, tenderness and juiciness were not significantly affected by starter culture and fermentation temperature. This indicates that the difference in the general acceptability of the fermented products is mainly due to the difference in perceived flavor/sourness. Highest general acceptability score (7.36±1.21; like moderately) was given to fermented carabeef inoculated with a combination of *L. plantarum* BS and *P. aciditactici* 3G3 starter culture, fermented at 10°C. However, this is not significantly different to the acceptability of the same treatment fermented at 32°C. Treatments inoculated with *P. acidilactici* 3G3 and fermented at both 32°C and 10°C

Table 3. Acceptability scores on the sensory properties of fermented carabeef as affected by starter culture and fermentation temperature.

Treatment	Acceptability scores					
	Color	Flavour	Off-flavor	Tenderness	Juiceness	General acceptability
Control	6.35±1.48[a]	7.43±2.53[a]	6.35±2.03[a]	2.06±0.93[b]	7.05±1.02[a]	2.67±0.93[d]
Fermented at 32°C						
T1	7.00±1.23[a]	5.27±2.25[b]	5.27±2.47[a]	7.05±1.29[a]	6.41±1.53[a]	5.27±1.72[bc]
T2	6.55±1.65[a]	5.32±2.40[b]	5.36±2.10[a]	6.77±1.63[a]	6.77±1.77[a]	5.18±2.08[c]
T3	6.82±1.37[a]	6.32±2.05[b]	6.18±2.30[a]	7.41±0.96[a]	6.77±1.48[a]	6.55±1.62[abc]
T4	6.68±1.39[a]	6.14±2.17[b]	6.00±2.43[a]	7.36±1.09[a]	7.00±1.69[a]	6.55±1.97[abc]
Fermented at 10°C						
T1	6.82±1.50[a]	6.77±2.04[ab]	6.18±2.34[a]	6.73±1.86[a]	6.32±1.99[a]	6.68±1.96[abc]
T2	6.82±1.50[a]	6.95±1.50[ab]	6.55±1.84[a]	7.18±1.40[a]	6.45±2.04[a]	6.68±1.78[abc]
T3	6.23±1.48[a]	6.50±1.77[ab]	6.00±2.09[a]	6.95±1.09[a]	6.41±1.81[a]	6.86±1.42[ab]
T4	6.73±1.49[a]	6.82±1.53[ab]	6.36±1.99[a]	6.36±1.84[a]	6.73±1.52[a]	7.36±1.21[a]

N=24; Means with a common letter superscript within a column are not significantly different at 5% level of significance based on Tukey's HSD. (T1= No Starter culture; T2= *L. plantarum* BS; T3= *P. acidilactici* 3G3; T4= Combination of *L. plantarum* BS and *P. acidilactici* 3G3).

also received high acceptability scores, not significantly different from the highest score given. The lowest score (5.18±2.08) for all fermented samples was obtained by the samples inoculated with *L. plantarum* 3G3 fermented at 32°C. This sample also got the lowest acceptability score for flavor/sourness as it was observed to be sourer than the other samples. Furthermore, results showed that the addition of starter culture rendered a more desirable fermented carabeef product as treatments inoculated with starter cultures had significantly higher acceptability scores than the uninoculated samples. This demonstrates the contribution of LAB to flavor generation and over-all product desirability.

The control non-fermented treatment received the lowest general acceptability scores from the panelists. The main factor that contributed to its low acceptability is tenderness. Non-fermented carabeef was perceived to be tough and therefore was disliked by the panelists.

3.4. Proximate composition

The treatment that scored the highest in terms of general acceptability in the sensory evaluation was subjected to proximate analysis. The treatment that had the highest acceptability score was the one inoculated with a combination of *L. plantarum* and *P. aciditactici* starter cultures and fermented at 10°C. Results are shown in Table 4.

Table 4. Proximate composition of carabeef samples before and after fermentation.

Proximate Component	Initial	Final
Moisture Content (%)	65.90 ±0.17[a]	64.07± 0.21[b]
Ash (%)	2.21±0.16[a]	2.55±0.08[a]
Crude Protein (%)	20.69±0.83[a]	19.62±0.46[a]
Crude Fat (%)	0.21±.08[b]	0.74±0.16[a]

Means with a common letter within a row are not significantly different at 5% level of significance.

The moisture content of the meat sample significantly ($p<0.05$) decreased after fermentation. There are reports that as pH of meat decreases, its moisture retention decreases, increasing its water holding capacity (WHC). The ash content of the sample was not significantly ($p>0.05$) affected by fermentation process. There was a decrease in the percentage of crude protein in the meat sample after fermentation although not significant ($p>0.05$). On the other hand, crude fat was found to increase significantly ($p<0.05$) after fermentation (0.21% to 0.74%). This increase in the final crude fat percentage is maybe due to moisture loss during fermentation. Although the same amount of fat is present, its percentage in the whole meat matrix increased because of the decrease in water content. It may also be caused by the variation in sliced meat used for sampling.

3.5. Fatty acid profile of fermented carabeef

The treatment that scored the highest in terms of general acceptability in the sensory evaluation was subjected to proximate analysis. The fatty acids present in large quantities in the meat samples include palmitic, stearic and oleic acid. Polyunsaturated fatty acids including linoleic and linolenic acids were also present in the meat samples**.** There was a significant increase ($p<0.05$) in the monounsaturated fatty acid (oleic) content in the meat samples after fermentation (from 27.64% to 37.56%). This increase can be due to endogenous and microbial lipase activity, generating fatty acids from lipid lipolysis (Casaburi *et al*., 2008). Higher amount of monounsaturated fatty acids is reportedly beneficial as researches have shown that they help lower bad (LDL) cholesterol levels. The presence of polyunsaturated fatty acids particularly linoleic and linolenic acids (before and after fermentation) also gives the product an advantage as polyunsaturated fats reduce

harmful LDL cholesterol, improving the cholesterol profile as well as lowering triglycerides in the body. Although there was a decrease in the amount of polyunsaturated fatty acids obtained in the study after fermentation (from 12.94% to 10.28%), the amounts obtained were still higher than the findings of Oliveros *et al*., (2007) wherein the total polyunsaturated fatty acid content of carabeef obtained was only 2.14%. This variability may be due to factors affecting the fat composition of meat including the age of animal and part of carcass used (Varela *et al*., 2004). The reduction in PUFA can be due to oxidative and hydrolytic reactions that occurred during fermentation. Long hydrocarbon chains and high unsaturation of PUFA make them more susceptible to oxidation and hydrolytic reactions (Chen *et al*., 2008).

Percent composition of saturated fatty acids decreased after fermentation due to the increase in the percentage of monounsaturated fatty acids in the whole fatty acid profile. Lower saturated to unsaturated fat ratio in foods is reportedly better, as monounsaturated and polyunsaturated fats are believed to promote good cholesterol (HDL) by helping move bad cholesterol to the liver, where it can be metabolized (Romero *et al*., 2013). In general, while the MUFA amounts of carabeef samples increased during fermentation, SFA and PUFA amounts decreased (Table 5).

Table 5. Fatty acid composition of carabeef samples before and after fermentation.

Fatty Acid	Composition (%)	
	Initial	Final
Myristic (C14:0)	3.17±1.15[a]	2.05±0.04[b]
Palmitic (C16:0)	30.34±4.54[a]	26.27±0.21[a]
Stearic (C18:0)	23.04±3.93[a]	22.74±2.20[a]
Oleic (C18:1)	27.64±4.68[b]	37.56±3.80[a]
Linoleic (C18:2)	12.88±2.74[a]	9.51±1.00[b]
Linolenic (C18:3)	0.06±0.69[a]	0.77±0.14[a]
Behenic (C22:0)	ND	ND
Tricosanoic (C23:0)	2.31±1.71[a]	1.10±0.62[a]
Lignoceric (C24:0)	ND	ND
∑SFA	58.86	52.16
∑MUFA	27.64	37.56
∑PUFA	12.94	10.28
Total	99.44	100

Values as means of duplicate samples. Means with a common letter within a row are not significantly different at 5% level of significance.

4. Conclusion

The effect of LAB starter cultures (*L. plantarum* BS and *P. acidilactici* 3G3) and fermentation temperature (32°C and 10°C) in the production of *pindang damulag* (fermented carabeef) were determined in terms of physicochemical, microbiological and sensory properties. The results of the study suggested that the addition of starter cultures has significant effect on the physicochemical, microbiological and sensory properties of fermented carabeef. Addition of starters, especially *P. acidilactici* 3G3 is a good modification in the production of *pindang damulag* as this resulted in a product with higher general acceptability and better microbial stability. These LAB strains can potentially be good inocula in producing a probiotic fermented carabeef, as their effect on the microbial and sensory properties of the product proved to be beneficial.

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