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Nutritional Content and Microbial Contamination of Fresh Cold and Frozen Bali Beef in Mambal RPH Production in Badung Regency, Bali Province

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***Abstract*---**This study aims to determine the nutrition and microbial contamination of fresh, chilled, and frozen Bali beef. This study used a completely randomized design (CRD) direct pattern a 3x7, with 3 treatments and 7 repetitions of Bali beef. The treatments were: (P1) meat stored at room temperature (27°C-35°C) for less than 1 day (fresh meat), (P2) meat stored at 0°C-4°C for 1 day -2 days (cold meat), (P3) meat stored at a minimum temperature of -18°C with a storage time of 1-7 days (frozen meat). The variables observed in this study were the nutritional content of meat, namely water content, protein, fat, ash and carbohydrates as well as pathogenic bacterial contamination, namely *Total Plate Count (TPC)*, *Colliform* and *E-Colli*. The results of this study showed that the nutritional content of water content and ash content in fresh, chilled and frozen meat had no significant effect. However, the protein content decreased significantly when the meat was frozen. The fat and carbohydrate content had the opposite result, namely, there was a significant increase when the meat was frozen. In terms of meat microbiological contamination on TPC, *Coliform* and *E-colli* variables, showed that frozen meat had the highest microbial population followed by fresh meat and cold meat had the lowest total pathogenic microbes.
***Keywords*---**Bali beef, chilled meat, fresh meat, frozen meat.

Introduction

Meat is one of the livestock commodities that have high nutritional value because apart from being a source of high-quality animal protein, meat is also a source of iron, vitamin B complex, fat, minerals and other substances that the body needs. The Provincial Government of Bali has issued Bali Governor Regulation Number 99 of 2018 concerning the Marketing and Utilization of Balinese Agricultural, Fishery and Local Industry Products. The Pergub also regulates the use of livestock products at least 30% of the needs of hotels and restaurants and at least 10% of the needs of the meat processing industry. To implement the Governor Regulation, especially for meat products, it is necessary to know the quality of meat in fresh, chilled or frozen form. This is very important to make a consumer consideration in choosing meat. The microbiological quality of meat is important to see aspects of meat safety, especially the contamination of pathogenic bacteria in meat which will later affect consumer health.

Meat is a food ingredient that is easily damaged or also known as perishable food, this is because meat contains quite good nutrients and has a favorable pH for microbial growth (Sarassati & Agustina, 2015), so common

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technologies are used in meat preservation such as refrigeration and freezing. Fresh meat, cold meat and frozen meat are types of meat that are grouped based on the physical condition of the meat. At present, many consumers consider the lack of quality of frozen meat compared to the quality of fresh meat and cold meat, making the market for selling frozen meat still lacking (Lu et al., 2022; Hu et al., 2022). This is supported by the results of research by Aritonang (2015), which suggests that as many as 70% of household consumers in the city of Padang prefer fresh meat because the quality of fresh meat is more guaranteed. Information from Balinese beef suppliers in Denpasar also complained about the difficulty of marketing frozen Bali beef in traditional markets (Sriyani et al., 2022). In addition to consumers' doubts about the hygiene of frozen meat, the lack of information about the quality of fresh, chilled and frozen beef also affects consumer tastes. Therefore, further research is needed to determine the nutritional content and microbial contamination of Bali beef under various fresh, chilled and frozen conditions (Qian et al., 2022).

Materials and Methods

The research material was a sample of beef loin in the Longissimus Dorsi (LD) muscle from a 3.5-year-old male Bali cattle with a slaughter weight of ±350 kg slaughtered at the Mambal Abiansemal Animal Slaughterhouse (RPH) Badung Bali. The samples consisted of 3 types, namely: fresh Bali beef, chilled Bali beef, and frozen Bali beef. The total number of samples used was 21 samples of loin Bali beef, each repeat sample weighing 250 g, divided into 7 samples of fresh Bali beef, 7 samples of chilled Bali beef and 7 samples of frozen Bali beef. For fresh meat stored at room temperature (27°C-35°C) with a storage time of less than 1-day, cold meat stored at cold temperatures (0°C-4°C) with a storage period of 2 days and frozen meat stored in the freezer at a minimum temperature of -18°C with a storage time of 7 days (frozen meat).

Experimental design

To find out the differences in nutrient content and microbial contamination of fresh, chilled and frozen Bali beef, it was carried out using a Completely Randomized Design (CRD) with a 3x7 pattern, namely with 3 treatments of Bali beef, namely:

- Treatment 1 (P1) = Bali beef stored at room temperature (27°C -35°C) for less than 1 day (fresh meat);
- Treatment 2 (P2) = Bali beef stored at 0°C - 4°C for 2 days (cold meat);
- Treatment 3 (P3) = Bali beef stored at -18°C for 7 days (frozen meat).

Each research treatment was repeated 7 times so that the total sample required was 3x7 = 21 samples.

Meat chemical quality test method
Water content

The water content was determined directly using an oven at 105^0C. First, the empty cup is dried in the oven at 105^0C for 15 minutes and cooled in a desiccator, then weighed. A total of 1.5 grams of sample was put in a cup that had been weighed and then dried in an oven at 105^0C for 3-4 hours. The cup containing the dried sample was then transferred to a desiccator, cooled for 30 minutes and then weighed. Drying was carried out until a constant weight was obtained. Calculation of water content can be calculated by the formula:

$$\% \text{ Moisture Content} = \frac{(\text{initial sample weight} - \text{final sample weight})(g)}{\text{initial sample weight}(g)} \text{ x } 100\%$$

Protein content

A total of 0.3 grams of sample was placed in a *vapodest* tube and added 1 grain of selenium catalyst and 5 ml of concentrated H2SO4, then digestion (heated to a boiling state) for 1.5 hours until the solution was clear. After cooling, 50 ml of distilled water and 20 ml of 40% NaOH were added and then distilled. The distillation results were collected in an Erlenmeyer flask containing a mixture of 20 ml of H3BO3 and 2 drops of pink-green bromine cresol. After the volume of the reservoir (distillate) became 100 ml and bluish, the distillation was stopped and the distillate was titrated with 0.1 N HCL until it turned pink. The same treatment was also applied to blanks. With this method, the crude protein content is obtained which is calculated by the formula:

$$\% \text{ Crude Protein Content} = \frac{(S-B)\ x\ 0{,}1\ x\ 14\ x\ 6{,}25}{W\ x\ 1000} \text{ x } 100\%$$

Note:

S: sample titrant volume
B: Blank titrant volume
W: dry sample weight

Fat level

Determination of fat content by the *Soxhlet* method. A sample of 2 grams of meat (A) was weighed and wrapped in filter paper and put in a tin, dried in an oven for 9 hours at 105^0C. The *soxtherm* tube is dried in an oven for 3 hours at 105^0C, then cooled in a desiccator and weighed (B). After drying, put the lead-containing sample into the *Soxtherm* tube, and fill the *Soxtherm* tube with 200 ml of n-Hexane until the sample is completely immersed. Extraction for 4 hours in the *Soxtherm* apparatus, then dry the *Soxtherm* tube in a forced oven for 15 minutes then dry it for 3 hours in a dry oven with a temperature of 105^0C, cool in a desiccator for 30 minutes, weigh the *Soxtherm* tube containing fat extract (C). The percentage of fat content is calculated as follows:

$$\text{Fat content (\%)} = \frac{\text{C} - \text{B}}{\text{A}}\ x\ 100\%$$

Note:

A: sample weight (grams)
B: *soxtherm* tube weight (grams)
C: *soxtherm* tube weight + fat extract (grams)

Ash content

The porcelain cup was heated in an oven at 100-105^0C for 30 minutes, then cooled in a desiccator and weighed until a constant weight was obtained. A total of 1 gram of meat sample was put into a porcelain cup and weighed, then burned until no longer smoking and roasted in a furnace at 600^0C for 3 hours until it was white and the weight was constant. Turn off the furnace, leave it for 12 hours then cool it in a desiccator for 30 minutes. After that, the sample was weighed.

$$\text{Ash content (\%)} = \frac{\text{weight of ash}}{\text{weight of sample}} \text{ x } 100\%$$

Microbiological quality test
Total Plate Count (TPC)

The steps for the TPC test were: smoothing the sample (beef) and weighing 5 grams of the sample. According to Waluyo (2008), the dilution stage starts with making a sample solution of 10 ml (a mixture of 1 ml/gram sample and 9 ml of peptone solution). Take 1 ml of the solution and put it in the next test tube so that the desired dilution is obtained. Then take the solution from the last 2 test tubes (10-7 and 10-8), pour it into the petri dish, then add the media in the form of agar and rotate it like number 8 so that the sample and media are evenly mixed and solidify, then the tube is incubated at 37ºC for 2 x 24 hours. The number of bacterial colonies can be calculated using the following formula:

$$\text{CFU} = \frac{\text{number of bacterial colonies}}{\text{dilution factor}} \times \textit{sampels poured}$$

Total coliform and escherichia coli

The method used to obtain total *Escherichia coli* and *Coliform* bacteria was the scatter method (Fardiaz, 1989) using EMBA media, namely as much as 5 grams of beef is put into an Erlenmeyer tube containing 0.1% peptone water solution with a volume of 45 ml so that a 10^{-1} dilution was obtained. This 10^{-1} dilution is then homogenized and diluted again by taking 1 ml through a pipette and then putting it into a test tube which already contains 9 ml of peptone solution so that 10^{-2} and 10^{-3} dilutions are obtained.

From the 10^{-1} dilution, 0.1 ml was taken using a sterile pipette and then poured on the surface of the solid EMBA media into a petri dish and then incubated at 37°C in an inverted state, and the results can be calculated after 24-48 hours. Planting was carried out at dilution levels of 10^{-1}, 10^{-2} and 10^{-3}. To count the growing bacterial colonies using the cup count method, namely by selecting the number of colonies that grew in Petri dishes ranging from 30 - 300 colonies (Fardiaz, 1989).

$$\text{Formula: Colonies/gram} = \text{Number of Colonies per cup x } \frac{1}{\text{dilution factor}}$$

Statistical analysis

Data on nutrient content and microbial contamination of the meat obtained were analyzed using variance. If there is a significant difference ($P<0.05$) between treatments, then the analysis is continued with Duncan's multiple range test (Steel & Torrie, 1993). The analysis was assisted by the SPSS 20 program. The microbial data obtained before being analyzed were first transformed into a log x form.

Results and Discussion

Chemical quality of fresh, chilled and frozen Bali beef

The results of the statistical analysis of testing the chemical content of meat (moisture content, protein content, fat content, ash content and carbohydrates) of fresh, chilled and frozen Bali beef can be seen in Table 1.

Table 1
Results of the nutritional content of fresh, chilled, and frozen Bali beef

Variable	Treatment (1)			SEM(2)
	P1	P2	P3	
Water Content (%)	72,18[a]	72,51[a]	71,94[a]	0,14
Protein Content (%)	24,26[a]	23,86[a]	23,14[b]	0,16
Fat Content (%)	1,97[b]	2,07[b]	2,32[a]	0,54
Ash Content (%)	1,10[a]	1,15[a]	1,10[a]	0,025

Note:
1. P1 : Bali beef in fresh condition
   P2 : Bali beef in cold condition
   P3 : Bali beef in frozen condition
2. SEM is “Standard Error of Treatment”

The results showed that there was no difference in meat water content ($P>0.05$) between fresh, chilled and frozen meat. Moisture content directly affects the quality of food ingredients. Moisture content is one of the determining factors for spoilage of food, including beef. Water contained in food is an excellent bacterial medium for the growth of pathogenic bacteria. The results of research on water content showed that the average moisture content of fresh, chilled and frozen meat ranged from 71-73 percent. This water content range is included in the normal category of beef moisture content according to the USDA, which is between 63-74 percent. Research Ernawati et al. (2018), obtained water content that was not significantly different between fresh, chilled and frozen beef in traditional markets and supermarkets. Research Diana et al. 2018 also obtained water content in fresh and thawed frozen meat statistically not significantly different with several thawing methods. A research study by Leygonie et al. (2012), reported that freezing causes water loss in meat because during freezing ice crystals form between and inside the meat fibers which physically damage the ultra-structure of meat fibers which causes there is no absorption of moisture into the intracellular space after the meat is thawed so that frozen meat has a lower water content. Ice crystals are formed by drawing water from the intracellular space into the intercellular space of the meat fibers. In this study, quantitatively, there was a decrease in the water content in frozen meat although not significantly different.

The results of this study showed a decrease in protein content in frozen meat ($P<0.05$). Protein Content Protein is a determining factor in determining the quality of a product in terms of product chemical properties. The results are

shown in Table 1. The protein content between fresh and chilled meat was not significantly different, but frozen meat thawed at room temperature showed a significant decrease (P<0.05) compared to fresh and chilled Bali beef. This is due to the arena in which frozen meat during thawing experiences drip (drip loss). The liquid or drips that come out during the thawing process has the potential to reduce protein levels because some nutrients dissolve and are lost with the water. This is following the opinion of Badrin et al. (2019), who stated that the low protein content can be suspected due to drip loss, namely the liquid that comes out of the product. Another thing is also due to the hydrophilic nature of the protein, so it may dissolve with water (drips). This is following the opinion of Wulandari & Rahayu (2014), who stated that the hydrophilic nature of protein allows these components to dissolve and disappear with drips. Protein content in this study ranged from 23-25%. This range of protein levels is still included in the range of good-quality meat. Soeparno (2015), stated that the protein content of meat ranged from 19-22%. Freezing meat is one way of preserving meat, namely by freezing meat below the freezing point of the liquid contained in the meat, the freezing point of meat at a temperature of -20ºC to -30ºC. Meat that was stored at a less than optimal temperature (≥--20ºC in this study (-18^{0}C) was probably produced by a slow freezing process. Slow freezing plus a length of storage may reduce the quality of frozen meat. Slow freezing will produce more liquid frozen meat (drip) including protein, which will reduce the quality of frozen meat, especially protein. Freezing speed determines the size of the ice crystals formed which will ultimately affect the quality of the product, in fast freezing soft ice crystals will form and if the freezing temperature decreases very quickly ultramicroscopic (very soft) ice crystals will form, the crystals formed will affect the amount of liquid which comes out when the meat is thawed again (drip), so it will affect the amount of liquid in the meat (Gracey, 1986). In cold meat, the protein content does not decrease significantly because the drip occurs very minimally.

The fat content in this study between fresh and chilled meat was not significantly different but in frozen meat there was a significant increase in fat content (P<0.05). The occurrence of drip during the thawing process causes water to come out of the meat but the fat contained in the meat cannot dissolve in water which causes the fat content to increase (Peck et al., 2005; Huang et al., 2022). The fat content of meat has a negative correlation with the water content of the meat, the higher the fat content, the lower the water content of the meat (Minish & Fox, 1979). In this study, the water content in frozen meat was quantitatively the lowest. This is not following the research of Diana et al. (2018), who stated that thawing in general still maintains the chemical quality of frozen meat under normal conditions. Kartika et al. (2016), also stated that at temperatures of ≥40ºC, some proteins will be denatured, but they have not been able to exceed the melting point of fatty acids, so only a small amount of fat is degraded.

The ash content of a food ingredient indicated the presence of inorganic mineral content in that food. Given the diversity of existing mineral sources, ash analysis is very important to determine nutritional quality and is often used as an indicator of food quality (Kanatt et al., 2005). In this study, the ash content of fresh, chilled and frozen meat did not show a significant difference (P>0.05). This is due to the possibility that what dissolves in the water that comes out of the meat/rips during the thawing process in frozen water is dissolved protein, not minerals. Especially proteins that are soluble in water. Dissolved proteins that may be lost with water during the thawing process include albumin and myoglobin proteins which are responsible for giving the red color to the meat. This is following the opinion of Aritonang (2015), which states that the components of water-soluble nutrients will also be lost with water during thawing, including albumin and myoglobin which is classified as sarcoplasmic proteins.

Microbiological quality of fresh, chilled and frozen Bali beef

The results of statistical analysis showed that the total plate count (TPC) of fresh Bali beef (P1) (2.3 x 105 cfu/g) chilled (P2) (1.8 x 105 cfu/g) and frozen (P3) (4, 9 x 108 cfu/g) was statistically significantly different (P<0.05). Tilapia TPC cold meat is the lowest followed by fresh meat and frozen meat. Total Coliform and E-Colli also showed the same trend as TPC where the lowest population was cold meat followed by fresh meat and the highest population was frozen meat (Table 2).

Table 2
Microbiological quality of fresh, chilled and frozen Bali beef

Variable	Treatment (1)		
	P1	P2	P3
TPC (*Total Plate Count*) (cfu/g)	2,3 x 10^{5b}	1,8 x 10^{5a}	4,9 x 10^{8c}
Total *Coliform* (cfu/g)	1,6 x 10^{5a}	1,5 x 10^{5a}	3,5 x 10^{8b}
Total *E-Colli* (cfu/g)	9,2 x 10^{3b}	6,1 x 10^{3a}	1,4 x 10^{7c}

Note:
1. P1 : Bali beef in fresh condition
   P2 : Bali beef in cold condition
   P3 : Bali beef in frozen condition

The results showed that the TPC, Coliform and E-Colli values showed significantly different results ($P<0.05$). The lowest value of microbial count was in cold meat, followed by fresh meat and the highest microbial count was in frozen meat (Huffman, 2002). The low microbial population in cold meat is because storage at cold temperatures can slow down the growth of the microbial population. Candradewi & Priyanto (2000), Storage at a cold temperature of 5^0C can slow down damage by microbes in meat. In fresh meat, the high microbial population is thought to be due to the influence of the treatment of the meat during the cutting process. Initial microbial contamination in meat can occur at the time of slaughter, the tools used for removing blood are not sterile and others. Fresh meat that is at room temperature tends to develop more microbes than meat at cold temperatures (Pearce et al., 2011; Joo et al., 2013). The high population of frozen meat is caused by drip that occurs when the meat is thawing. The increase and decrease in the number of microbes can be influenced by the length of freezing, where each meat has a different moisture content, the higher the water content is not chemically bound, the more microbes will grow (Wulandari & Rahayu, 2014). The crystallization process forms ice in frozen meat and the water is chemically bound, so the water cannot be used by microorganisms, but if the thawing process is carried out again, the water can vice versa be used by microorganisms to reproduce (Buckle et al., 1987). Therefore, the role of water is very influential in the growth of microorganisms, so the material that has been thawed must be processed immediately to prevent the growth of more microorganisms. The results of this study are following the results of Dewi (2012), study which also found a higher TPC population in frozen meat than fresh meat.

Conclusion

The results of this study showed that the nutritional content of water content and ash content in fresh, chilled and frozen meat had no significant effect. However, the protein content decreased significantly when the meat was frozen. On the microbiological contamination of meat on the variable TPC, Coliform and E-colli showed that frozen meat had the highest microbial population followed by fresh meat and cold meat had the smallest total pathogenic microbes.

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