PROTEIN PROFILE BASED ON SDS-PAGE IN SOATED BEEF WITH VARIED CONCENTRATIONS OF GAMBIR (Uncariagambir roxb) AND VARIATIONS IN TIME IMMERSION

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Abstract . Meat is a food ingredient that has nutritional value in the form of high protein and contains a complete and balanced amino acid composition. The presence of microbes in beef requires proper handling, storage or processing to prevent the activity of microorganisms. One of the natural antibacterial ingredients that contain flavonoid compounds is found in gambier. The research objective was to determine the protein profile of beef soaked with gambier powder with variations in concentration and soaking time using the SDS-PAGE method. The design of this study was a descriptive study with the object of research being beef soaked in gambier powder with a concentration of 4%, 6%, 8% and soaking time of 30, 45, 60 minutes respectively. The results showed that there were 19 protein subunits in the control whereas in the treated sample there were 6 - 13 protein sub units, the sub units that did not occur protein denaturation when given treatment were 66 kDa, 33 kDa, 21 kDa, 6-10 kDa. From the results of the research conducted, it was shown that there was an effect of beef soaked in gambier powder with high concentrations and a long time to produce a lower total protein than beef soaked with low concentrations and a shorter soaking time.

Keywords: Beef, Gambir, protein profile, SDS-PAGE

1 INTRODUCTION

Meat is a food that has nutritional value in the form of high protein and contains a complete and balanced composition of amino acids. Beef is an animal food ingredient that is popular with all levels of society because of its delicious taste. According to Soeparno (2009), in general beef contains about 75% water, with a range of 68% -80%, about 19% protein (16% -22%), 1% minerals and about 2.5% fat (1.5%). %-13.0%) (Júnior et al., 2022).

The presence of microbes in beef requires proper handling, storage, or processing to prevent the activity of microorganisms in beef, so efforts need to be made to maintain the durability of the beef. Flavonoid compounds are phenolic compounds that have been known to inhibit the growth of bacteria in beef. One of the natural antibacterial ingredients that contains flavonoid compounds is found in gambier (Wu, 2020).

Gambier is a product from the gambier plant (Uncaria gambir Roxb) which contains functional compounds which are included in the group of polyphenolic compounds, one of the elements of which is catechin. Catechin compounds can damage bacterial cell membranes or walls, thereby disrupting cell permeability. Analysis of the protein profile of beef was carried out by separating the proteins into simpler molecules using SDS-PAGE electrophoresis techniques (Ferreira de Oliveira et al., 2022).

Based on research by Sari., 2017, the soaking treatment in gambier with a concentration of 6% for 45 minutes was able to reduce the microbial population which resulted in a quality profile (water activity, protein content, texture, color and ALT) of smoked catfish that was better than without soaking gambier (Sari1 et al., 2017) .Tumangger et al (2017) also conducted research on the effect of the concentration of natural preservatives from gambier leaf extract on the quality (total microbes, pH of tofu, pH of soaking water, water content, ash content, protein content, color, aroma, taste and texture) of tofu during storage. The research results showed that the best results were at a concentration of 0.25% gambier leaf extract with a storage period of 2 days (Dosunmu et al., 2020).

Research conducted by Tumangger (2017) shows that gambier is able to reduce microbial growth in food ingredients. But this has never been done on beef, with the high public interest in beef on the market, it is necessary to test the protein profile of beef with the addition of gambier powder. Beef protein profile analysis was carried out by separating proteins into simpler molecules using SDS-PAGE electrophoresis techniques (lee, et al , 2020).

2 RESEARCH METHODS

The research design used was descriptive research. The independent variable in this study was soaking beef with gambier powder with a concentration of 10% / , 4%, 6%, 8% with soaking times of 30, 45, 60 minutes respectively. The dependent variable in this research is the

protein profile of beef. The object of this research is beef soaked in gambier powder with a concentration of 4%, 6%, 8%／with a soaking time of 30, 45, 60 minutes. This research was carried out at the Biomolecular laboratory at Muhammadiyah University, Semarang and the Biotechnology laboratory at Gajah Mada University, Jogjakarta. The tools used are: drying oven, blender, microtube, micropipette, glass beaker, Erlenmeyer, electrophoresis chamber, power supply, centrifuge, rotator, mortal cup and spectrophotometer. The materials used are gambier plants made in powder form, beef, 30% polyacrylamide, TEMED, APS 10%, SDS 10%, 1.5 M Tris pH 8.8 and 6.8, coomassie brilliant blue (CBB) staining, 10% glacial acetic acid, biorad assay, sample buffer, and protein marker (Mazur et al., 2023)

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Research procedure: the gambier plant is boiled, the boiled plant is squeezed to extract the sap from the gambier plant, air is felt on the sediment to make it solid. The precipitate that has been solid and dry is then crushed into powder. Soaking beef with gambier powder at a concentration of 4% w/w; 6% w/b; 8% w/w, namely 20 grams of beef, then add 0.8 each; 1,2; 1.6 grams of gambier powder is then soaked for 30, 45 and 60 minutes.

Fresh beef samples and those that have been soaked in gambier powder at a concentration of 4% w/v for 30, 40 and 60 minutes are ground in a mortar cup or blender. Once smooth, weigh 10 grams each, add 35-50 ml of PBS 1x Ph 7.4 and then homogenize. This mixture was put into a conical tube and homogenized using a vortex, then centrifuged at 3000 rpm for 20 minutes at 40C. meat that has been centrifuged then the supernatant is taken, the supernatant is protein. The protein concentration obtained was measured using a speaktrophotometer. Preparation of a 2000 µl blank using a 2 µl microtube was carried out using 1798 µl distilled water plus 200 µl Biorad reagent and 2 µl sample supernatant. The absorbance is read at a wavelength of 595 nm. Next, samples of beef soaked in gambier powder at a concentration of 6% w/v and 8% w/v were carried out in the same way as for beef soaked in gambier powder at a concentration of 4% w/v for 30, 40 and 60 minutes (Nowakowski et al., 2014).

The protein separation method using SDS-PAGE prepared glass plates, spacers, combs that had been cleaned with detergent and 70% alcohol for gel printing. Sperating gel and stacking gel solutions were made using a printer. The sperating and stacking gel solution was added in 15 ml for 2 pairs of 12% solution glass plates as a separating gel,

then butanol was added to cover the surface of the solution sufficiently, waited 30-60 minutes until polymerization occurred.(Dao et al., 2023). Next, the gel that has undergone polarization is mounted on a mini protein B biorat, then added to it is a pH 8.3 buffer electrode solution, stacking 5% gel, 5 ml of solution for 2 pairs of glass plates. The sample is prepared, the sample is added with 5x sample buffer with a ratio of 4:1(v/v) after which the mixture is heated for 2 minutes in boiling water, then immediately placed on ice. The sample is then ready to be inserted into the gel, after which an electric current is applied with a voltage of 100 volts until bromphenol blue comes out from the bottom of the gel (Qu et al., 2020) (Dao et al., 2023).

The gel was taken and then stained with CBB (Comassie Brilliant Blue) 0.1% R-250 for 30-60 minutes until the protein bands were stained. Next, to remove the color from the gel which does not contain protein, it is given a destaining solution. The destaining solution is changed 3-4 times until the gel looks clean, then to determine the molecular weight (BM) of the desired protein, the Retardention Factor (RF) is calculated and plotted on a logarithmic graph and the Rf is a protein marker whose molecular weight is known. Calculations are carried out by measuring the distance of movement of protein bands using the formula (Sri Darmawati, 2017)

Calculations were carried out by measuring the distance of movement of protein bands using the Laemmli 1970 formula.

$$Rf = \frac{the\ distance\ the\ protein\ moves\ from\ its\ starting\ point\ (distance\ traveled)}{distance\ of\ movement\ of\ the\ protein\ band\ from\ the\ initial\ site\ (total)}$$

3 RESULTS AND DISCUSSION

The results of measuring the total absorbance of beef protein coated with gambier powder at concentrations of 4%, 6% and 8% w/v with varying soaking times of 30, 45, 60 minutes are shown in Table 1.

Table 1. Total beef protein spectrophotometrically

Gambir powder concentration ($\%^{b}/_{v}$)	Immersion time (minutes)			
	0	30	45	60
0	1,838 µg/µl	-	-	-
4	-	1,124 µg/µl	0,805 µg/µl	0,509 µg/µl
6	-	1,102 µg/µl	0,742 µg/µl	0,543 µg/µl
8	-	1,088 µg/µl	0,708 µg/µl	0,560 µg/µl

Based on Table 1. shows the total protein in beef that was not given treatment (control) showed a greater total protein compared to beef that was soaked with variations in the

concentration of gambier powder with variations in soaking time. The total protein in beef is affected by the high concentration and soaking time of gambier powder. Beef soaked in gambier powder with high concentrations and a long time can produce lower total protein than beef soaked with low concentrations and a shorter soaking time. This is because the catechin compounds contained in gambier powder are play a role in hydrolysis reactions, namely reactions that involve the element of water in substrate-specific bonds (Pane et al., 2018)

Analysis of the protein profile of beef that has been smeared with gambier powder was carried out using the SDS-PAGE method which can be seen in Figures 1 and 2.

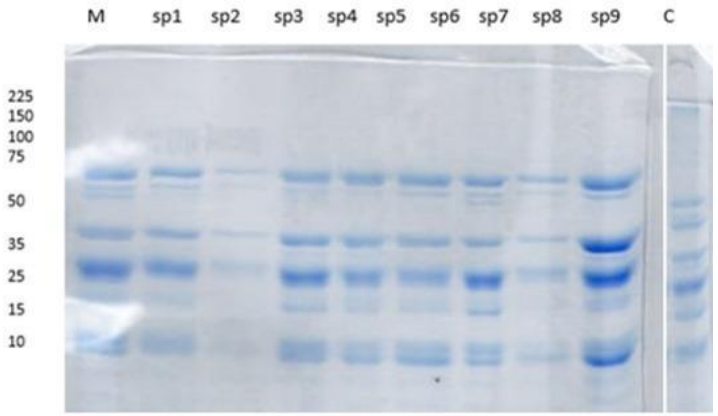

Fig. 1. Results of beef protein bands smeared with gambier powder

Information : sp 1 (sample concentration 4% with soaking time 30)
sp 2 (sample concentration 4% with soaking time 45)
sp 3 (sample concentration 4% with soaking time 60)
sp 4 (sample concentration 6% with soaking time 30)
sp 5 (sample concentration 6% with soaking time 45)
sp 6 (sample concentration 6% with soaking time 60)
sp 7 (sample concentration 8% with soaking time 30)
sp 8 (sample concentration 8% with soaking time 45)
sp 9 (sample concentration 8% with soaking time 60)

Analysis of the protein profile of beef that has been coated with gambier powder was carried out using the SDS-PAGE method which can be seen in the picture

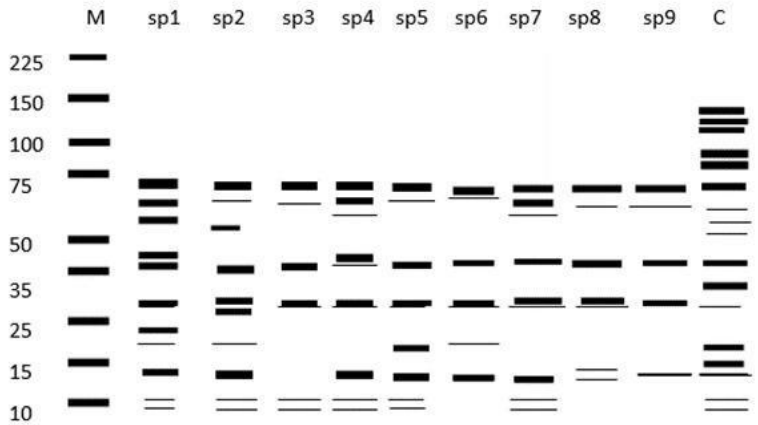

Fig. 2. Visualization of protein band representation of beef covered with powder

The protein profile of beef shown in Figure 1 shows the difference between untreated beef (control) and meat soaked with varying concentrations of gambier powder and varying soaking times. The beef soaked in gambier powder was then separated with 12% SDS-PAGE and stained with 2% Comassie Brilliant Blue (CBB), then pressed and calculated Rf (Retardation factor) of each protein subunit (bend protein) using the following formula (Nowakowski et al., 2014)

$$Rf = \frac{the\ distance\ the\ protein\ moves\ from\ its\ starting\ point\ (distance\ traveled)}{distance\ of\ movement\ of\ the\ protein\ band\ from\ the\ initial\ site\ (total)}$$

Table 2. Retardation factor markers

BM marker	Mileage (Cm)	Total distance (cm)	Rf (X)	Log BM (Y)
225	1,1	5,5	0,2	2,04762
150	1,4	5,5	0,2545	1,9333
100	1,8	5,5	0,3272	1,7808
75	2	5,5	0,3636	1,7044
50	2,5	5,5	0,4545	1,5138
35	3	5,5	0,5454	1,3231
25	3,5	5,5	0,6363	1,1325
15	4,3	5,5	0,7818	0,8273
10	4,7	5,5	0,8545	0,6748

Protein molecular weight is measured using a standard protein whose molecular weight is known by comparing the retardation factor (Rf) value using the formula (Fatchiyah et. al 2011):

Table 3. Analysis results and sample molecular weight

Sampel code	**Molecular Weight (kDa)**	***Band* protein**
C	65, 60, 50, 27, 23, 21, 10, 8, 6, 5 and 4 kDa 145, 133, 122, 102, 93, 33 dan 14 kDa	11 mayor 8 minor
Sp1	66, 55, 51, 33, 21, 13, 8, 6 and 5 kDa 30, 19, 14, 5 and 4 kDa	8 mayor 5 minor

Sp2	66, 33, 21, 19, 8, dan 6 kDa	6 mayor
	55, 13, 5 and 4 kDa	4 minor
Sp3	66, 33 and 21 kDa	3 mayor
	55, 19, 8 and 6 kDa	4 minor
Sp4	66,55, 33, 21,8 and 6 kDa	6 mayor
	51, 30, 19, 5 and 4 kDa	5 minor
Sp5	66, 33, 21, 13, 8 dan 6 kDa	6 mayor
	55, 19, 5 and 4 kDa	4 minor
Sp6	66, 33, 21, 8 and 6 kDa	5 mayor
	55,19 dan13 kDa	3 minor
Sp7	66, 55,33, 21,8 and 6 kDa	6 mayor
	51, 19, 5 and 4 kDa	4 minor
Sp8	66, 33 and 21 kDa	3 mayor
	55, 19, 8 and 8 kDa	4 minor
Sp9	66, 33 and 21 kDa	3 mayor
	55, 8 and 6 kDa	3 minor

Based on the information in Table 3, there were 19 protein subunits in the control while in the treated beef there were 13 to 5 protein subunits.

The process of soaking beef with varying concentrations and varying soaking times for gambier powder shows the loss of protein bands, this occurs because the presence of catechin compounds which are combined with protein compounds will form protein catechin complexes which can combine to form a phenol oxidation property. Oxidation of phenol in catechins allows amino acid and protein reactions to occur which can inhibit the activity of proteolytic enzymes (Gazda et al., 2020)

The results obtained from the research will show that beef samples without treatment (control) and soaked with varying concentrations of gambier powder and variations in soaking time can be seen in Table 10. There were 19 protein subunits in the control while in the treated samples there were 6 to 13 protein subunits. The decrease in the number of major and minor bands in each treatment was due to the high concentration used for each soaking time. There were several subunits that did not experience protein denaturation when treated with gambier powder with varying concentrations and varying soaking times, namely Albumin (66 kDa) , Tropomyosin (33 kDa), Trypsin inhibitors (21 kDa), Metallothionenin (6-10 kDa) (Lee et al., 2020)

In general, people do cooling and freezing to maintain the freshness and quality of beef .However, the dry extract from Gambir leaves is able to preserve rending because it contains polyphenolic compounds or catechins which are included in flavonoid compounds. The addition of flavonoid compounds contained in gambier can inhibit the growth of bacteria in beef (Ho et al., 2023).

Changes in the protein profile in beef without treatment showed high major and minor bands, whereas in beef with treatment there was a gradual loss of protein bands, this indicates protein destruction which is characterized by the loss of small subunits. The protein profile can be measured from the number of N-terminals in the sample that are bound to the Biorad protein Assay (BPA) so that it will produce a color that will be

absorbed by UV light in speaktrophotometry using a wavelength of 595nm to produce the absorbance of the sample (Ferreira de Oliveira et al., 2022)

4 CONCLUSION AND RECOMMENDATION

Based on the results of research on beef soaked with gambier powder, it shows that the best profile results are at a concentration of 4%w/w with a soaking time of 30 minutes. This states that the addition of gambier powder to beef will break the peptide bonds in meat protein into simpler micromolecules so that the meat will become softer. The difference in the concentration of gambier powder will show different results, namely the higher the concentration of gambier powder, the amount of protein in the meat will be denatured. For future researchers, they can carry out further research by soaking using other parts of the gambier plant and for the community to tenderize meat, they can use the gambier plant.

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