



Physicochemical Characteristics of Beef Meatballs at Various Post Mortem Phases with the Addition of Seaweed Flour (*Gracillaria* sp.)

Ririh Wahyuningtyas Hapsari¹, Ratna Yulistiani^{2*}.

¹²Food Technology Departement, Faculty of Engineering, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya, Indonesia.

ARTICLE INFO

Article History:

Received: 10 June 2024

Final Revision: 09 July 2024

Accepted: 10 July 2024

Online Publication: 11 July 2024

KEYWORDS

meatballs, post-mortem, pre-rigor, rigor mortise, post-rigor, seaweed

CORRESPONDING AUTHOR

*E-mail: ratna.tp@upnjatim.ac.id

ABSTRACT

The post-mortem phase of meat influences the physicochemical quality of the beef meatballs produced. The post-mortem phase is the phase that meat goes through after slaughter. The post-mortem phase is divided into 3, namely prerigor, rigor mortis, and post-rigor. Seaweed (*Gracillaria* sp.) can be used as a natural chewy meatball. This research aims to determine the best treatment combination between the post-mortem phase of meat and the addition of seaweed flour on the physicochemical characteristics and preferences of beef meatballs. This research used a completely randomized design with a factorial pattern with 2 factors with two replications. The factor I is the post-mortem phase (pre-rigor, rigor mortise, and post-Rigo). Factor II is the addition of seaweed flour (3%, 4% and 5%). The observation data were analyzed using ANOVA, if there were significant differences, the Duncan Multiple Range Test was carried out at the 5% level. The best treatment results were pre-rigor beef treatment with the addition of 5% seaweed flour, producing meatballs with characteristics of 69.05% water content, 2.93% ash content, 1.55% fat, 12.49% protein, 12.92N elasticity level, 47.22% water holding capacity, Color preference level 4 (like really), aroma 4.07 (like really), taste 4.17 (like really), and texture 4.50 (like really).

1. INTRODUCTION

1.1 Research Background

Meatballs are a typical Indonesian food that is loved by many people. Meatballs are wet food products in the shape of spheres or other shapes obtained from beef or chicken. [1]. Generally, meatballs use the main raw material in the form of fresh meat that has not experienced rigor mortis. After the slaughtering process, a stiffening process (rigormortis) will occur in the carcass and meat due to the presence of residual energy in the form of adenosine triphosphate which results in the contraction of the myofilament actin and myosin. The post-mortem phase consists of three phases, namely the pre-rigor, rigormortis, and posterior phases. The prerigor phase is a process that occurs less than 7 hours after slaughter or immediately after the livestock is slaughtered (death) in cattle and sheep, the rigormortis phase occurs after 7-15 hours and the postrigor phase occurs after 15 hours after slaughter in livestock [2].

Pre-rigor meat (occurs less than 7 hours after slaughter) is usually used to produce chewy and compact meatballs. This is because beef in the prerigor phase has high water-holding capacity so the ability of meat protein to bind and retain water is high. Apart from that, the meatballs' elasticity is also stimulated by the change in beef from the pre-rigor phase to rigormortis during grinding, mixing, grinding, molding, and boiling.

The rigormortis phase (which occurs 7 to 15 hours after slaughter) is a process where the meat changes to become stiff and lose its flexibility. In this phase, the muscle tissue becomes hard and stiff, due to the decrease in ATP in the muscles. The time required for rigor mortis to develop depends on the amount of ATP available at the time the animal dies. The amount of ATP available is related to the amount of glycogen available at the time the animal dies. In livestock that are tired or stressed and lack rest before slaughter, this will result in a low ATP supply so that the rigor mortis process will occur quickly. Likewise, high temperatures when livestock are slaughtered will accelerate the depletion of ATP due to breakdown by the ATPase enzyme so rigor mortis will occur quickly.



The post-rigid phase occurs 15 hours after the meat is slaughtered. In this phase, the cathepsin enzyme will disintegrate the myofilaments, eliminate the adhesion force on the meat, and loosen the protein structure of the auto fibers so that the meat will soften again. In this phase, the products of glycolysis accumulate, resulting in a buildup of lactic acid so that the pH of muscle tissue is low, accumulation of ATP breakdown products, formation of flavor and aroma precursors, increased water holding capacity, and re-softening of muscle tissue. Apart from the post-mortem phase, the addition of chewing ingredients can also affect the characteristics of the meatballs produced.

The addition of chewing ingredients is usually used to improve the texture of meatballs because meat sold in the market is thought to have passed the prerigor phase. Examples of the types of chewing agents used in making meatballs are sodium tripolyphosphate, carboxymethyl cellulose, and calcium chloride. To reduce the use of chewing agents that come from chemicals, we can use seaweed (*Gracilaria* sp.) as a natural chewing agent that is safer and comes from non-chemical ingredients [3]. *Gracilaria* sp. is a seaweed that produces primary metabolites in the form of hydrocolloid compounds called agar. Agar has very strong gel strength [4]. Apart from containing hydrocolloids, Reff [5] states that seaweed is a marine commodity that is rich in nutrients. Fiber from seaweed and fiber from other food ingredients that enter the body causes regular bowel movements so that it can prevent obesity, coronary heart disease, colon cancer, and diabetes. The main ingredients of seaweed are agar, alginic acid, and carrageenan.

Research on the use of seaweed flour in making beef meatballs shows that the addition of seaweed pulp as much as 30%, 40%, and 50% can increase elasticity, water-holding capacity, and organoleptic preferences [6].

Research on the effect of the post-mortem phase of meat with the addition of seaweed flour on beef meatballs is not yet known. Therefore, in this study, research was conducted on the effect of the post-mortem phase of meat with the addition of seaweed flour on the physicochemical characteristics of the beef meatballs produced.

1.2 Literature review

1.2.1 Beef Meatballs

Meatballs are one of the processed meat products that are popularly consumed by the public because they taste delicious are relatively affordable and contain high protein. The nutritional content of meatballs consists of a minimum protein content of 9%, a maximum fat content of 2%, a maximum water content of 70%, and a maximum ash content of 3% [7].

The meat usually used is beef, chicken, and fish, while the filler used is tapioca flour. The quality of meatballs is determined by their water-holding capacity, elasticity, and nutritional content. Good quality meatballs have high water holding capacity, this shows that water is bound to protein and free water is captured in the meat cells. The level of elasticity of good quality meatballs is that they can break due to pressure and have high nutritional content [8]. The nutritional composition of beef per 100 grams can be seen in Table 1.

Table 1. The nutritional composition of beef per 100 grams of ingredients

Nutrients	beef
Water (gram)	66,0
Protein (gram)	18,8
Energy (K)	207,0
Fat (gram)	14,0
Calcium (mg)	11,0
Iron (mg)	2,8
Vitamin A(SI)	30,0

The addition of carrageenan can increase the yield of meatballs because carrageenan has gel-forming properties. Gel formation in carrageenan is the process of forming a three-dimensional network by primary molecules stretched over the volume of the gel formed by combining a certain amount of water in it so that it can increase the yield value in meatballs. Apart from that, carrageenan is a fraction that can form a gel in water and is reversible, that is, it melts if heated and forms a gel again when cooled. The heating process at a temperature higher than the gel formation temperature will cause the carrageenan polymer in the solution to become random. If the temperature is lowered, the polymer will form a double helix structure and if the temperature continues to decrease, these polymers will be strongly cross-linked as the helix shape increases, aggregates will be formed which are responsible for the formation of a strong gel [9].

1.2.2 Seaweed

Seaweed is one of the biological resources that is very abundant in Indonesian waters. Various types of seaweed, such as *Gelidium* sp., *Gracilaria* sp., *Gelidiella* sp., and *Hypnea* sp. are types of seaweed that have economic value [10]. According to data from the Ministry of Maritime Affairs and Fisheries, national seaweed production is growing at an average of 11.8% per year, whereas in 2017, national seaweed production was recorded at 10.8 million tons. The export value of seaweed also experienced a growth of 3.09% per year [11]. Seaweed is suitable as a food ingredient and is beneficial for health because it contains fiber, amino acids, low fat, carbohydrates, minerals, and vitamins [12]. The chemical composition of *Gracilaria* sp can be seen in Table 2.

Table 2. Chemical composition of *Gracilaria* sp

Nutrients	Beef
Calories (kcal)	312
Protein (g)	1.3
Fat (g)	1.2
Carbohydrates (g)	83.5
Fiber (g)	2.7
Ash (g)	4
Calcium (mg)	756.0
Phosphorus (mg)	18
Iron (mg)	7.8
Sodium (mg)	115.0
Potassium (mg)	107.0
Thiamine (mg)	0.01
Riboflavin (mg)	0.22
Niacin (mg)	0.2

In making meatballs, the addition of 15% seaweed slurry can increase the elasticity and water absorption of the meatballs produced [13]. Water binding capacity is not only determined by myofibril proteins but also by water-binding components [14].

1.2.3 Post-mortem phase

The post-mortem phase consists of three phases, namely the pre-rigor, rigor mortise, and post-rigor phases. The pre-rigor phase is a process that occurs less than 7 hours after slaughter or immediately after the livestock is slaughtered (death) in cattle and sheep, the rigormortis phase occurs after 7-15 hours and the post-rigor phase occurs after 15 hours after slaughter. in livestock. The use of pre-rigor-stage meat produces chewy meatballs compared to meat from rigor mortis and post-rigor treatments [15]. In the pre-rigor phase, the pH of the meat decreases because there is no blood circulation to the muscle tissue, then in the rigor mortise phase the meat muscles will experience stiffness due to the interaction between actin and myosin enzymes, and in the post-rigor phase the meat will experience softening again because the cathepsin enzyme is active due to the pH which goes down. The cathepsin enzyme is a proteolytic enzyme that can loosen the protein structure of muscle fibers.

1.3 Objective

This research aims to determine the effect of the post-mortem phase of meat and the addition of seaweed flour on the physicochemical characteristics of meatballs.

2. MATERIALS AND METHODS

2.1 Materials and Tools

The main ingredient used in this research was beef obtained from the Surya Pegirian Surabaya Slaughterhouse with meat samples in pre-rigor condition (less than 7 hours) and meat samples in the rigor mortise phase (7-15 hours). Other ingredients used include seaweed (*Gracillaria* sp.), tapioca flour, salt, eggs, spices such as shallots, garlic, pepper, granulated sugar, and ice cubes.

Materials used for chemical analysis include distilled water, hexane solvent, HCL, selenium-Mix, concentrated H_2SO_4 , CaO 5%, boric acid (H_3BO_3), and indicators MR+MB, NaOH, phosphate buffer, Termamyl, Protease, and Amyloglucosidase.

The tools used are Soxhlet extraction tool, Kjeldahl flask, Shibaura Aw meter, desiccator, oven, cabinet dryer, autoclave, aluminum cup, porcelain cup, furnace, measuring cup, pH meter, hotplate, condenser, Erlenmeyer flask, measuring flask, pipette burette, and filter paper and aluminum foil. Apart from that, the tools used are a food processor, scales, spoons, pans, large bowls, basins, and gas stoves.

2.2 Research Design and Analysis

The research design used in this study was a Completely Randomized Design (CRD) with a factorial pattern with two factors and 2 replications. The treatment used was the post-mortem phase of meat (Pre-rigor, Rigor Mortis, Post-rigor) and the addition of seaweed flour (3%, 4%, 5%). The observation data was analyzed using Analysis of Variance (ANOVA), if there were significant differences, a further test was carried out. If there were significant differences, a further test was carried out using

DMRT with a level of 5%. The treatment combinations studied can be seen in Table 3.

Table 3. Treatment of research experiments

The mortem phase of meat	Seaweed Flour		
	B1 (3%)	B2 (4%)	B3 (5%)
A1 (Pre-rigor)	A ₁ B ₁	A ₁ B ₂	A ₁ B ₃
A2 (Rigor Mortis)	A ₂ B ₁	A ₂ B ₂	A ₂ B ₃
A3 (Post-rigor)	A ₃ B ₁	A ₃ B ₂	A ₃ B ₃

2.3 Research Implementation

2.3.1 Making *Gracilaria* sp. seaweed flour.

Wet seaweed is washed thoroughly using clean water, then soaked in 48% NaOH, 98% H_2SO_4 , and 12% NaOCl for 30 minutes each. After soaking in this acid, it was immediately washed again until the pH was neutral. The second washing process aims to wash and neutralize the pH of seaweed that has been soaked in an acid solution. pH measurements are carried out with pH paper. If the washing process is not optimal, it will affect the gel strength of the resulting agar powder. Then it is boiled in water for 4 hours and drained. After that, the seaweed is dried using a cabinet dryer machine at a temperature of 40°C for 10 hours. After drying, the dried seaweed is ground using a blender until smooth and sieved using a 60-mesh sieve. Sifting is done so that the seaweed flour produces a smooth texture.

2.3.2 Making meatballs with the addition of *Gracilaria* sp. seaweed flour.

Meatballs using the basic ingredient is 100 grams of beef with post-mortem pre-rigor, rigor mortise, and post-rigor phases. Next, cut the beef into 3x3 cm sizes and grind and mix using a chopper. The supporting ingredients added are 30 grams of tapioca flour, 3 grams of salt, 1 egg, 3 grams of shallots, 6 grams of garlic, 3 grams of pepper, and sugar. 1 gram and 15 grams of ice cubes. After that, printing is carried out with a diameter of 3cm. The final stage is boiling at a temperature of 100°C.

2.4 Observation

2.4.1 Raw Material Analysis

The raw materials used are beef and seaweed flour. The analysis carried out on beef is water holding capacity, water content, pH, yield, and organoleptic.

2.4.2 Product chemical analysis

Chemical analysis parameters include analysis of water content, ash content, protein content, fat content, and water holding capacity.

2.4.3 Physical analysis

Analysis parameters include elasticity using the texture analyzer method.

2.4.4 Sensory analysis

Organoleptic testing was carried out on beef meatball samples. In this organoleptic test, 30 panelists assessed the texture, aroma, color, and taste of beef meatballs.

3. RESULTS AND DISCUSSION

3.1 Raw Material Analysis

Analysis of post-mortem beef raw materials includes analysis in the form of water content, WHC, and pH which can be seen in Table 4.

Table 4. Results of analysis of beef raw materials

Beef	Parameter		pH
	Moisture Content (%)	Water Holding Capacity (%)	
Pre-rigor	75.51 ± 0.18	87.43 ± 0.06	6.8 ± 0.14
Rigor mortis	70.68 ± 0.24	83.92 ± 0.18	6.5 ± 0.28
Post rigor	66.15 ± 0.25	71.13 ± 0.24	5.8 ± 0.00

The research results in Table 4 show that the water holding capacity, water content, and pH of meat decrease as the rigor mortis phase occurs. The moisture content of beef meatballs during post-mortem treatment decreased, this occurred because water came out of the carcass. This is following research by ref [16] that meat at the 0th hour (pre-rigor) has a water content of 71.27% and will decrease further at the 12th hour to 69.67. This is because the evaporation and release of weep (fresh meat fluid) increases with the length of the post-mortem process.

The water-holding capacity of beef meatballs during post-mortem treatment decreased, this was due to the post-mortem acidity of the meat. A lower pH causes a lower water holding capacity. This change in pH is caused by the cessation of the respiration process after slaughter and causes glycolysis to occur and the production of lactic acid. This is following the ref's statement [17] that after the slaughter process, blood circulation in the animal will stop. This will cause the blood's function as an oxygen carrier to stop. When the respiration process stops, the glycolysis reaction will occur and result in the production of lactic acid, which will continue with a series of biochemical and chemical changes such as changes in meat pH, changes in protein solubility, changes in water holding capacity, changes in muscle tissue. The cessation of the respiration process also causes a decrease in the amount of ATP (adenosine triphosphate) in meat tissue which functions as an energy source [18]. This is characterized by an increase in stiffness in the muscle tissue known as the rigor mortis phase.

Analysis of seaweed flour raw materials includes analysis in the form of yield, water content, ash content, fat content, protein content, and organoleptic (color, aroma, taste, and texture) which can be seen in Table 5

Table 5. analysis of seaweed flour

Parameter	Result
Yield (%)	4.20 ± 0.03
Moisture Content (%)	5.37 ± 0.27
Ash Content (%)	13.73 ± 0.16
Fat Content (%)	0.56 ± 0.30
Protein Content (%)	6.75 ± 0.40
Organoleptic :	
- Color	White
- Aroma	Not scented
- Flavor	Tasteless
- Texture	Normal

The results of the analysis of seaweed flour produced a yield of 4.2%, water content of 5.37%, fat content of 0.56%, and protein content of 6.75% with a white color, tasteless and aromatic, and a normal texture. The low yield produced is due to the high water content in seaweed so if wet seaweed is dried into flour, it produces a small mass of flour. This is supported by Ref [19] that the yield of seaweed flour is very small due to the high water content of seaweed. A weight of 5000g of seaweed produces 210g of flour, so the resulting yield percentage is 4.2%. Apart from that, there are differences between water content, ash content, fat, and protein content, this is due to differences in the place of growth and age of the plants used in this research and the literature. This is following ref [20] that the difference in results is thought to be influenced by the place where the plant grows or the variety used.

3.2 Chemical Analysis

3.2.1 Moisture Content

Based on the results of observations in Table 6, it can be seen that there is a significant interaction ($p \leq 0.05$) between the post-mortem phase treatment of beef and the treatment with the addition of seaweed flour on the water content of beef meatballs and each treatment has a significant effect. This shows that the use of beef with different rigormortis phases and the addition of seaweed flour as a meatball ingredient affects the water content contained in the meatballs. The average value of water content of beef meatballs with post-mortem treatment and the addition of seaweed flour can be seen in Table 6

Table 6. The average value of water content of beef meatballs with the influence of the post-mortem phase and the addition of seaweed flour

Treatment	Moisture Content (%)
A ₁ B ₁	62.82 ± 0.06 ^f
A ₁ B ₂	65.56 ± 0.12 ^g
A ₁ B ₃	69.05 ± 0.08 ^h
A ₂ B ₁	43.42 ± 0.03 ^a
A ₂ B ₂	45.06 ± 0.08 ^b
A ₂ B ₃	47.68 ± 0.41 ^c
A ₃ B ₁	50.29 ± 0.13 ^d
A ₃ B ₂	54.88 ± 0.09 ^e
A ₃ B ₃	55.16 ± 0.06 ^e

Note: Average values followed by different letters indicate significantly different values ($p \leq 0.05$).

Table 6 shows that the average water content of the meatballs produced is between 43.42 – 69.05%. The post-mortem pre-rigor phase treatment with the addition of 5% seaweed flour produced the highest water content, namely 69.05%, while the post-mortem rigormortis phase treatment with the addition of 3% seaweed flour produced the lowest water content, namely 43.42%. The water content of meatballs in this study is following the quality standard SNI 01-3818-1995 which states that the maximum water content standard in meatballs is 70%. A graphic of the relationship between post-mortem phase treatment and the addition of seaweed flour can be seen in Figure 1.

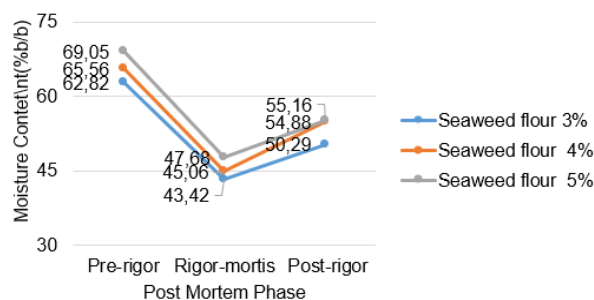


Figure 1. The relationship between post-mortem phase treatment and the addition of seaweed flour on the water content of beef meatballs

Figure 1 shows that the longer the post-mortem phase process can reduce the water content, however, the higher the seaweed flour can increase the water content of the meatballs. This is because the rigor mortis phase of the meat will experience stiffness and will not have space to bind water. The addition of seaweed flour can also increase the water content because the hydrocolloid content in seaweed can absorb water so it can cause an increase in the water content of the meatballs produced. Ref [15]. This loss of water content is caused by the proteins that play a role in meat, namely the actin and myosin proteins, which have interacted to form actomyosin. Reff [13] explained that the hydrocolloid content in seaweed slurry can bind water. Hydrocolloid compounds can bind water, so they can suppress the process of water coming out of the product during cooking [21].

3.2.2 Ash Content

Ash content is the mineral content of the product. Based on observations of the ash content in Table 7, shows that the average ash content of the meatballs produced is between 2.27 – and 2.93%. The post-mortem rigormortis phase treatment with the addition of 5% seaweed flour produced the highest ash content, namely 2.93%, while the prerigor post-mortem phase treatment

with the addition of 3% seaweed flour produced the lowest ash content, namely 2.27%.

In meatballs with meat in the rigor mortis phase, the ash content increases, but the ash content decreases again when the meat reaches the post-rigor phase. The addition of seaweed flour can increase the ash content of the meatballs produced. The high or low ash content can be influenced by several factors, namely the type of material used, if the percentage of water content in the material is high, it can reduce the percentage of ash content produced. This is supported by Reff's research [15] that increasing the water content in meatballs will cause a decrease in the ash content concentration.

The decrease in ash content when using post-rigor meat is thought to be caused by the process from rigor mortis to post-rigor where fresh meat water (weep) comes out of the meat which is accompanied by the release of minerals in the meat, therefore the ash content is lower. This is following [14] during the withering process from the 0th hour to the 24th hour, the release of Ca and Na and the absorption of K into the sarcoplasm by myofibril proteins due to a decrease in pH. Ca and Na are then dissolved. With weep and drip, with longer withering, the mineral content may decrease.

The use of seaweed flour can increase the ash content of meatballs. Seaweed flour has an ash content of 17.09%, so the more seaweed flour you add, the more ash content the beef meatballs produced will increase. As explained by ref [22], the ash content shows the amount of minerals contained in the material, so the higher the mineral content in the raw material, the higher the ash content in the meatball product. Reff [23] also said that the addition of more raw materials with high ash content can increase the ash content of the products produced.

3.2.3 Protein Content

Based on the results of statistical analysis, it can be seen that there is a significant interaction ($p \leq 0.05$) between the post-mortem phase of beef and the treatment with the addition of seaweed flour on the protein content of beef meatballs and each treatment.

Table 7. Results of chemical analysis of beef meatballs

Chemical Analysis	Treatment								
	A ₁ B ₁	A ₁ B ₂	A ₁ B ₃	A ₂ B ₁	A ₂ B ₂	A ₂ B ₃	A ₃ B ₁	A ₃ B ₂	A ₃ B ₃
Ash Content (%)	2.27 ± 0.03 ^a	2.33 ± 0.01 ^b	2.43 ± 0.02 ^c	2.59 ± 0.01 ^e	2.70 ± 0.02 ^f	2.93 ± 0.01 ^g	2.46 ± 0.01 ^{cd}	2.49 ± 0.01 ^d	2.53 ± 0.02 ^e
Proteins Content (%)	12.15 ± 0.02 ^c	12.28 ± 0.06 ^f	12.49 ± 0.05 ^g	11.86 ± 0.04 ^c	11.98 ± 0.01 ^d	12.18 ± 0.05 ^e	11.42 ± 0.06 ^a	11.74 ± 0.01 ^b	11.82 ± 0.02 ^c
WHC (%)	43.11 ± 0.02 ^f	44.82 ± 0.29 ^g	47.22 ± 0.05 ^h	29.98 ± 0.19 ^a	33.12 ± 0.06 ^b	35.63 ± 0.06 ^d	34.10 ± 0.02 ^c	35.59 ± 0.69 ^d	37.06 ± 0.09 ^e

Note: Average values followed by different letters indicate significantly different values ($p \leq 0.05$)

Table 7 shows that the average protein content of meatballs produced is between 11.42 – 12.49% and shows that the longer the meat takes to be processed (going through the pre-rigor to post-rigor phase) the protein content produced decreases, the more flour is added. seaweed, the higher the protein content produced. This is because as rigor mortis progresses, there is an enzymatic breakdown of protein in the beef used. Reff [8] protein levels decreased with each treatment and stage of decreasing freshness. The rigor phase occurs when myofibril proteins contract to form actomyosin, shorten the distance between sarcomeres, reduce the distance between proteins, apply pressure

such as grinding or heating causing some to denature. In the post-rigor phase, shrinkage occurs due to contraction, the sarcomeres shrink and protein denaturation occurs. Reff's research [15] shows that there is a decrease in the protein content of meatballs as the post-mortem process of the beef used continues. The addition of seaweed to making meatballs can increase the protein content of the meatballs produced. This is because seaweed contains high protein, namely 16.83% [6] so the more seaweed flour added can increase the protein content in food. This is supported by Reff [6] that seaweed has a protein content of

16.83%, so the more seaweed added can increase the amount of protein in the meatballs produced.

3.2.4 Water Holding Capacity (WHC)

Based on the results of the analysis, it can be seen that there is a significant interaction ($p \leq 0.05$) between the post-mortem phase of beef and the treatment of adding seaweed flour to the WHC of beef meatballs which can be seen in table 7.

The average WHC value of the meatballs produced is between 29.98 and 47.22%. The pre-rigor post-mortem phase treatment with the addition of 5% seaweed flour produced the highest WHC, namely 47.22%, while the post-rigor post-mortem phase treatment with the addition of 3% seaweed flour produced the lowest WHC, namely 29.98%.

When the meat reaches the post-rigor phase, the pH of the meat will decrease further, making the cathepsin enzyme active which causes the water-holding capacity to increase again. This is following [17] that the rigor mortis phase of meat is very stiff and has no room to bind water because of the strong cross-linking bonds between actin and myosin in meat muscle tissue. However, by decreasing the pH value, the cathepsin enzyme becomes active so that the water-holding capacity will increase again. [24] added that, when the pH of meat is low, it contains a lot of lactic acid, so the reactive groups of proteins are reduced and this will cause more water to be released so that the water-holding capacity is low. Low water holding capacity causes meat to release a lot of water, the meat becomes soft, and wet and the color of the meat will look pale. The results of this research are supported by Reff's research [25] where meat stored for 6, 24, and 48 hours produces a decreasing water-holding capacity.

3.2.5 Fat Content

Based on the results of statistical analysis, it can be seen that there is no significant interaction ($p \geq 0.05$) between the post-mortem phase treatment of beef and the treatment with the addition of seaweed flour on the fat content of beef meatballs. The post-mortem phase treatment had a significant effect on the fat content of the meatballs, while the addition of seaweed flour did not provide a significant difference. The average value of the fat content of beef meatballs with post-mortem treatment can be seen in Table 8.

Table 8. Average value of fat content of beef meatballs with post-mortem phase treatment

Post Mortem Phase Treatment	Fat Content (%) $\pm$ SD
Pre rigor	1,55 $\pm$ 0,00 ^a
Rigormortis	2,35 $\pm$ 0,00 ^c
Postrigor	2,04 $\pm$ 0,01 ^b

Description: Average values followed by different letters indicate significantly different values ($p \leq 0,05$)

The observation results (Table 8) show that the post-mortem rigormortis phase treatment produces the highest fat content, namely 2.35%, while the post-mortem pre-rigor phase treatment produces the lowest fat content, namely 1.55%. The fat content increases during the pre-rigor to rigormortis phase, this is because when the meat is in the rigormortis phase, the meat will lose water in the ingredients, and postrigor the water content will increase, so the percentage of fat content can increase as the water content decreases. This is following Reff's research [15] that beef

meatballs that produce the highest fat content come from meat in the postrigor, prerigor, and postrigor phases, respectively. This is because, during rigormortis, the meat will lose water due to the interaction of actin and myosin proteins so the percentage of fat content will increase. However, during post-rigor, the cathepsin enzyme will be active and disintegrate the myofilament muscle tissue, eliminating the adhesion force between muscle fibers and loosening the protein structure so that water can re-enter, so that the percentage of fat content decreases. The average value of fat content in meatballs with the addition of seaweed flour can be seen in Table 9

Table 9. Average value of fat content of beef meatballs with the addition of seaweed flour

Seaweed Flour (%)	Fat Content (%)
3	1.98 $\pm$ 0.98 ^a
4	1.98 $\pm$ 0.98 ^a
5	1.98 $\pm$ 0.98 ^a

Description: The average value followed by different letters indicates a significantly different value ($p \leq 0.05$)

Table 9 shows that the addition of seaweed flour does not have a significant effect on the fat content of the meatballs produced. This is because seaweed flour has a low-fat content, namely 0.41%, so adding seaweed flour has no real effect.

This is following Reff [26] that the addition of seaweed flour as much as 0%, 3%, 5%, and 7% does not make a difference in the fat content of the product produced.

3.3 Physical Analysis

Physical analysis of beef meatballs influences the post-mortem phase of meat and the addition of seaweed flour includes the level of chewiness. Based on the results of the analysis of variance, there was a significant interaction ($p \leq 0.05$) between the post-mortem phase treatment of beef and the treatment with the addition of seaweed flour on the level of chewiness of the beef meatballs and each post-mortem treatment and the addition of seaweed flour can be seen in Table 10.

Table 10. The average value of the firmness level of beef meatballs with the influence of the post-mortem phase and the addition of seaweed flour

Treatment	Firmness Level (N)
A ₁ B ₁	11.52 $\pm$ 0.03 ^f
A ₁ B ₂	12.29 $\pm$ 0.08 ^g
A ₁ B ₃	12.92 $\pm$ 0.09 ^h
A ₂ B ₁	9.49 $\pm$ 0.08 ^{cd}
A ₂ B ₂	9.60 $\pm$ 0.06 ^d
A ₂ B ₃	10.17 $\pm$ 0.06 ^e
A ₃ B ₁	8.64 $\pm$ 0.06 ^a
A ₃ B ₂	9.11 $\pm$ 0.08 ^b
A ₃ B ₃	9.43 $\pm$ 0.00 ^{bc}

Note Average values followed by different letters indicate significantly different values ($p \leq 0.05$).

Table 10 shows that the average level of firmness of the meatballs produced is between 8.64 and 12.92N. The pre-rigor post-mortem phase treatment with the addition of 5% seaweed flour produced the highest level of elasticity, namely 12.92N, while the post-mortem postrigor phase treatment with the addition of 3% seaweed flour produced the lowest elasticity level, namely 8.64N. A graph of the relationship between post-mortem

phase treatment and the addition of seaweed flour to the level of hardness and meatballs can be seen in Figure 2.

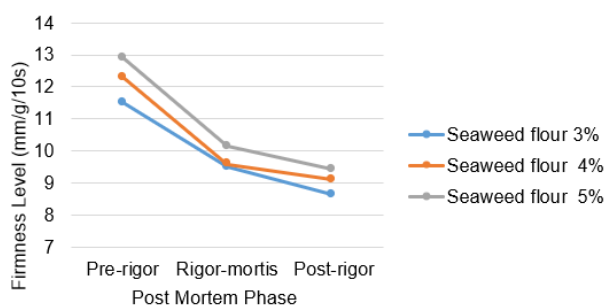


Figure 2. The relationship between post-mortem phase treatment and the addition of seaweed flour on the level of chewiness of beef meatballs

Figure 2 shows that the longer the meat takes to go through the post-mortem phase, the meatballs' chewiness can decrease, but the addition of seaweed flour can increase the chewiness level. This is because meat in the pre-rigor phase has a higher ability to bind water than meat in the rigor mortis and post-rigor phases. This ability to bind water causes the texture to become more chewy and compact. In the rigor mortis phase, meat has experienced cross-linking between actin and myosin which causes the meat to become stiff. This is supported by Reff [17] that the strong cross-linking bonds between actin and myosin in meat muscle tissue make the meat very stiff or not chewy. Reff [8] said that chewiness is related to low water holding capacity, meat with low water holding capacity causes a lot of water to come out during cooking, and less gel is formed. When the meat reaches the post-rigor phase, the pH of the meat drops further, making the cathepsin enzyme active which causes the water-holding capacity to increase again.

The addition of seaweed flour (*Eucheuma cottonii*) can increase the elasticity of the meatballs produced. This condition is caused by the carrageenan content found in seaweed which has the property of filling food because it can bind water [27]. This is following research [13] in his research, namely that making quail meatballs with the addition of seaweed can increase the elasticity of the resulting meatballs. Quail meatballs with the addition of 15% seaweed pulp had a higher average elasticity value than meatballs without the addition of seaweed. The increase in elasticity value is because seaweed contains carrageenan which has a high water-binding ability, so it can increase the elasticity value of processed products. Carrageenan can bind with protein and water, thereby increasing the elasticity of meatballs. Carrageenan binds with proteins, carbohydrates, and water, thereby improving cutting characteristics. Carrageenan can bind water and can bind meat particles, causing a chewy texture [28].

3.4 Sensory Analysis

To determine the effect of the post-mortem phase of meat and the addition of seaweed flour including the level of chewiness on the sensory properties of beef meatballs by distributing questionnaires to 30 panelists using the hedonic test method for aroma, color, taste and texture parameters. It can be seen in the following table:

The observation results in Table 11 show that the post-mortem phase with the addition of seaweed flour had a significant effect ($p \leq 0.05$) on the color of the beef meatballs produced. The

average meatball color scoring value ranged between 3.27-4.10. The prerigor meat treatment had a higher average preference than meatballs using rigormortis and postrigor meat, whereas with the addition of seaweed flour the average color value was almost the same. This is because during storage from the prerigor phase to rigormortis and postrigor, the meat which was originally fresh red in color changes to red-brown due to exposure to air, thus affecting the color of the meatballs produced. The addition of seaweed flour also did not have a significant effect on the taste of the meatballs, because there was little seaweed flour added. This is following Reff [29] that the color of freshly cut beef that has not been exposed to air is purplish red, then if it has been exposed to air for approximately 15-30 minutes it will change to a bright red color. The bright red color will change to red-brown or brown if the meat is left exposed to air for a long time.

Table 11. Average hedonic test value of beef meatballs

Treatment	Color	Aroma	Taste	Texture
A ₁ B ₁	4.10	4.10	4.20	3.77
A ₂ B ₁	4.00	4.07	4.20	4.20
A ₃ B ₁	4.00	4.07	4.17	4.50
A ₂ B ₁	3.70	3.60	4.20	3.63
A ₂ B ₂	3.77	3.63	4.17	3.70
A ₂ B ₃	3.73	3.63	4.07	3.83
A ₃ B ₁	3.30	3.60	3.93	3.10
A ₃ B ₂	3.27	3.67	4.13	3.17
A ₃ B ₃	3.30	3.70	4.23	3.37

Note : Scale 1 = Very Dislike; 2 = Dislike; 3 = Neutral; 4 = Like; 5 = Very Like

Table 11 shows that the post mortem phase with the addition of seaweed flour had no significant effect ($p \geq 0.05$) on the aroma of the beef meatballs produced. The average scoring value for the aroma of meatballs ranged between 3.60-4.10 in the prerigor phase of meatballs producing an aroma that was very liked by the panelists, however, there was a decrease in the use of the rigormortis and post rigor phases, the addition of seaweed flour did not significantly differ in the aroma produced. This is because beef that is still fresh (rigormortis) still has the typical aroma of fresh beef, while beef in the rigormortis and post-rigor phases produces a less fresh aroma. This research follows research conducted by Reff [29] that the aroma of fresh meat is better in beef that has not undergone storage (immediately processed) than beef that has been stored for 10 hours. Reff [30] Ministry of Agriculture, 2009 explains that the aroma of fresh meat does not smell sour/rotten, but has a distinctive aroma of fresh meat. The aroma of meat is influenced by the type of animal, feed, age of the meat, gender, fat, length of time, and storage conditions.

Table 11 shows that the post-mortem phase with the addition of seaweed flour did not have a significant effect ($p \geq 0.05$) on the taste of beef meatballs, indicating that the post-mortem phase of beef and the addition of seaweed flour did not have a difference in the results of liking the meatballs produced. This is because the taste of the meatballs is influenced by the spices used, in this study the same percentage of spices was used for all parameters, and the amount of seaweed flour added was small so that it did not affect the taste of the resulting meatballs.

Table 11 shows that the post-mortem phase with the addition of seaweed flour has a significant effect ($p \leq 0.05$) on the texture

of beef meatballs. It shows that the post-mortem phase of beef and the addition of seaweed flour shows that meatballs with prerigor meat are more preferred than meatballs made from beef. with the rigor mortis and post rigor phases, as well as the addition of more seaweed flour, can increase panelists' preferences. This is because meatballs from prerigor meat are chewier and juicier than meatballs from rigormortis and postrigor meat. This is following research conducted by Ref. [29] that fresh meat without withering produces a very favorable texture than meat that has withered for 6, 8, and 10 hours, this is because the texture of fresh meat is more chewy and juicy than withered beef, because The longer it withers, the drier the texture of the meat will look. According to Ref [30] fresh meat has a supple texture, dense and not stiff, when pressed with the hand, the massage marks return to their original shape. In Ref's research [31] the water holding capacity decreased from 6 hours withering to 10 hours withering. This is in line with the results of this study where beef that was withered for 10 hours had a rougher and drier meat texture but was not yet classified as damaged.

4 CONCLUSION

The conclusion of this research shows that the post-mortem phase of meat and the addition of seaweed flour have a real interaction on the physicochemical characteristics of beef meatballs. The best combination of treatment for beef meatballs is found in sample A1B3, namely the pre-rigor meat phase and the addition of 5% seaweed flour to produce meatballs with characteristics of 69.05% water content, 2.43% ash content, 1.55% fat, 12.49% protein, 12.92N elasticity level, water holding capacity 47.22%, color favorability level 4, aroma 4.07, taste 4.17, and texture 4.50 resulting in better physicochemical characteristics and a higher favorability level than other samples.

REFERENCE

- [1] Aulawi, T dan Ninsix. "Sifat fisik bakso daging sapi dengan bahan pengenyal dan lama penyimpanan yang berbeda". *Jurnal Peternakan*. Vol 6(2). 2009.
- [2] Hafid, H. dan Syam, A. "Pengaruh Aging dan Lokasi Otot terhadap Kualitas Organoleptik Daging Sapi". *Buletin Peternakan* Vol. 31 (4). 2017
- [3] Untoro, N.S., Kusrahayu dan Setiani, B.E. "Kadar Air, Kekenyalan, Kadar Lemak dan Citarasa Bakso Daging Sapi dengan Penambahan Ikan Bandeng Presto (*Channos channos* forsk). *Animal Agriculture Journal* Vol. 1(1). 2012
- [4] Anggadiredja, A., Syahrini, N dan Irawati. "Rumput Laut: Pembudidayaan, Pengolahan dan Pemasaran Komoditas Perikanan Potensial", Jakarta: Penebar Swadaya, 2009.
- [5] Lutony, T. "Tanaman Sumber Pemanis", Jakarta: Penebar Swadaya, 1993.
- [6] Princestasari, L.D dan Amalia, L. "Formulasi Rumput Laut *Gracilaria* sp. Dalam Pembuatan Bakso Daging Sapi Tinggi Serat dan Iodium. *Jurnal Gizi Pangan* Vol. 10 (3). 2015.
- [7] SNI 01-3818 "Bakso Daging Sapi". Jakarta: Badan Standarisasi Nasional. 1995
- [8] Kusnadi, Bintoro dan Al Baarri. "Daya Ikat Air, Tingkat Kekenyalan dan Kadar Protein pada Bakso Kombinasi Daging Sapi dan Daging Kelinci", *Jurnal Aplikasi Teknologi Pangan*. 2012.
- [9] Chandra, M. "Pemanfaatan Teknologi Ultrafiltrasi untuk memproduksi karaginan", Bogor : Institut Pertanian. 2001
- [10] McHugh, D. J. "A Guide to The Seaweed Industry", *Jurnal Food and Agriculture Organization of The United Nations*. 2003
- [11] Kementerian Kelautan dan Perikanan (KKP). *Produktivitas Perikanan Indonesia*. 2019
- [12] Amaranggana dan Larasati. "Pelayanan Informasi Obat yang Efektif dari Beberapa Negara untuk Meningkatkan Pelayanan Farmasi Klinik", *Jurnal Farmaka*. 2017
- [13] Anggreini, F.N., Suryaningsih, L dan Putranto, S.W. "The Effect of Seaweed Addition (*Eucheuma cottonii*) on Physical Quality and Acceptability of Quail Meatball". 2020
- [14] Nurul, F., Willy, P. W dan Ela, T.S. "Penambahan Jamur Tiram Putih (*Pleurotus ostreatus*) dan Konsentrasi Penstabil terhadap Karakteristik Patty Ikan (*Pangasius* Sp). Thesis. UNPAS. 2017
- [15] Heriawan, Sandiah, N dan Hafid, H. "Pengaruh Fase Rigormotis terhadap Kualitas Kimia Bakso Sapi" *Jurnal Ilmiah Peternakan Halu Oleo* Vol 2 No 4. 2020.
- [16] Widati, A.S. "Pengaruh Lama Pelayuan, Temperatur Pembekuan dan Bahan Pengemas Terhadap Kualitas Kimia Daging Sapi Beku". *Jurnal Ilmu dan Teknologi Hasil Ternak*. Vol 3(2). 2008
- [17] Lonergan, E. H., Zhang, W dan Lonergan, S. M. "Biochemistry of post mortem muscle – lesson on mechanisms of meat tenderization". *Journal Meat Science*. 2010.
- [18] Lonergan, E., Zhang, H.W. dan Lonergan, S.M. "Biochemistry of Postmortem Muscle Lessons on Mechanism of Meat Tenderization. *Meat Science* Vol 86. 2010
- [19] Anggraini, P. R. "Pemanfaatan Rumput Laut (*Eucheuma cottonii*) menjadi Roti Tinggi Serat dan Yodium". *Jurnal Arsip Gizi dan Pangan*. 2018.
- [20] Amannu, F.N. dan Susanto. "Pembuatan Tepung Mocaf di adura (Kajian Varietas dan Lokasi Penanaman terhadap Mutu dan Rendemen". *Jurnal Pangan dan Agroindustri* Vol 2 (3). 2014
- [21] Herawati, H. "Potensi Hidrokoloid sebagai Bahan Tambahan Pangan pada Produk Pangan dan Non Pangan Bermutu". *Jurnal Litbang Pertanian* Vol 37 (1). 2018
- [22] Amanda, A. "Aktivitas Antioksidan dan Karakteristik Organoleptik Minuman Fungsional The Hijau (*Camellia sinensis*) rempah instan. Skripsi Fakultas Pertanian Institut Pertanian Bogor. 2009
- [23] Wardani, M. K., Jariyah dan Anggreini, R. A. "Karakteristik Biskuit Tepung Mocaf dan Tepung Kulit Biji Kedelai Kuning". *Jurnal Ilmu Pangan dan Hasil Pertanian*. 2022.
- [24] Wahyuni, D., Arisuteja, S. Sandi, S. dan Yosi, F. "Pengaruh Suplementasi Probiotik dalam Ramsum Terhadap Kualitas Fisik Daging Itik. *Sains Peternakan : Jurnal Penelitian Ilmu Peternakan*. Vol 14 (2). 2016
- [25] Taringan, M.M., Wibowo, A dan Ardhani, F. "Pengamatan Perubahan Sifat Fisik Otot Semitendinosus Sapi Pasca Penjualan Selama Masa Simpan Dingin". *Jurnal Peternakan Lingkungan Tropis*. 2020.
- [26] Agusman, Siti, N. K. dan Murdinah. 2014. "Penggunaan Tepung Rumput Laut *Eucheuma cottonii* pada pembuatan Beras Analog dari Tepung Mocaf". *Jurnal JPB Perikanan*. 2014.
- [27] Lawrie, R. A. "Ilmu Daging Terjemahan: Parakassi, A dan Y. Amulia". *Jurnal Meat Science*. Jakarta : UI Press 2003
- [28] Ayadi, M. A., A. Kechaou, I, Makni dan Attia, H. "Influence of Carrageenan Addition on Turkey Meat Sausages Properties". *Journal Food Engineering*. 2009
- [29] Sinaga, dkk. "Kualitas Organoleptik Daging Sapi Bali yang Dilayukan dengan Lama Waktu yang Berbeda". "Majalah ilmu peternakan. 2021
- [30] Deptan. "Pemilihan dan Penanganan Daging Segar". www.pustakadeptan.go.id/agritek/lip50019.pdf. 2011
- [31] Poety, M.K., Sriyani, N.L.P. dan Oka, A.A. "Kualitas Fisik Daging Sapi yang Dilayukan Secara Tradisional". *Majalah Ilmiah Peternakan* Vol. 24(2). 2021.