EVALUATION OF THE MYOFIBRILLAR FRAGMENTATION INDEX: EFFECT OF PROTEIN DETERMINATION AND ABSORBANCE READINGS

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Abstract – Different laboratories use distinct protocols for Myofibrillar Fragmentation Index (MFI) analysis. Hence, this work aimed to test the MFI buffer and sodium hydroxide (NaOH) solutions on the protein and MFI values and the materials used in absorbance readings on the MFI values in aged beef. Five Nellore castrated males were slaughtered and steaks from *Longissimus lumborum* muscles were aged for 1, 7, and 14 days post-mortem (pm). The meat samples were homogenized to obtain suspense myofibrils, which were diluted in either MFI buffer or NaOH for protein determination. Amounts of suspense myofibrils based on the protein values were used to quantify MFI values through the absorbance readings with cuvette, glass tube, and microplate. Higher protein values in NaOH than in MFI buffer were found at 1 and 7 days pm. In MFI buffer, the meat samples at 7 and 14 days pm had higher protein values than at 1 day pm. Within all the times pm, MFI buffer showed higher MFI values than NaOH. Meat samples at 7 and 14 days pm had higher MFI values than at 1 day pm within NaOH. MFI values from cuvette and glass tube did not differ, while MFI values from microplate were the lowest. In conclusion, MFI buffer may underestimate protein values and overestimate MFI values. The cuvette and glass tube may be used for absorbance readings regarding MFI values, whereas the microplate not.

Key Words – methodology, MFI, and protocol.

I. INTRODUCTION

Since 70s decade, the first works attempting to link tenderness and myofibrillar proteolysis of meat were carried out [1,2,3]. The methodology that quantifies the amount of myofibrillar proteolysis was referred to as Myofibrillar Fragmentation Index (MFI). It consists of three steps: extraction of the myofibrillar protein, determination of protein by biuret reaction, and absorbance readings of the suspense myofibrils at a certain protein concentration. Until nowadays, this methodology has been used to describe meat quality [4,5,6]. The original method describes the use of MFI buffer for the dilution of the suspense myofibrils during the protein determination and to use cuvette for the absorbance readings of the suspense myofibrils during the MFI values determination [2,3]. However, current protocols used in some laboratories have guided to use sodium hydroxide (NaOH) solution for the dilution of the suspense myofibrils during the protein determination and to use glass tubes for the absorbance readings of the suspense myofibrils during the MFI values determination. The NaOH solution would be used to unfold the protein structures and to standardize the protein determination by dissociating disulphide bonds [7,8]. On the other hand, the glass tubes would be used to optimize the time of the absorbance readings, avoiding transferring the suspense myofibrils to the cuvette in each reading. There is no work testing if these different ways of carrying out the MFI analysis may affect the final MFI values. Therefore, this work aimed to evaluate the effect of different dilution solutions on the protein and MFI values and materials for absorbance readings on the MFI values in meat from *Bos indicus* cattle. Additionally, samples taken across

the times post-mortem (pm) were used to check the response pattern of the values and possible interactions.

II. MATERIALS AND METHODS

Five Nellore-type castrated males with average weight of 464 ± 55 kg and average age of 25 months were used. Steaks from the *Longissimus lumborum* muscles were aged for 1, 7, and 14 days for the protein and MFI determination. The extraction of myofibrillar protein was conducted in duplicate. Meat samples (2 g) were homogenized with 20 mL of MFI buffer (100 mM KCl, 20 mM KH_2PO_4, 20 mM K_2HPO_4, 1 mM EDTA, and 1 mM $MgCl_2$) for three times (30 s each burst with 30 s rest) on 18,000 rpm using a Tecnal Turratec TE 102 homogenizer. The remainder of the extraction was performed according to previous works [2,3]. The step of the protein determination in the suspensions obtained after samples extraction was performed in two different ways. The same suspension (50 μL) was diluted in either MFI buffer (150 μL) or NaOH 1 M (150 μL) solutions into an eppendorf and vortexed. Next, each one of the diluted suspension (60 μL) was placed into two wells of the ELISA microplate (96 wells) before adding biuret reagent (240 μL). After 30 minutes under darkness, the diluted suspensions readings were performed at 540 nm in a Thermo Scientific Multiskan FC microplate photometer. Bovine Serum Albumin (BSA) was used to establish a protein concentration standard curve. The protein values obtained from each diluted (MFI buffer and NaOH) suspension were used to make a 0.5 mg protein/mL solution using appropriate quantities of the suspension and MFI buffer in a final volume of 300 μL (ELISA microplate), 3 mL (cuvette), and 5 mL (glass tube). The absorbance readings of the suspensions were also performed at 540 nm using three different spectrophotometers for three different materials: Thermo Scientific Multiskan FC microplate photometer for microplate, Marte Spectro 560 Visible spectrophotometer for cuvette, and Unico S1205 Visible spectrophotometer for glass tube. In turn, the absorbance values were multiplied by 200 to obtain the MFI values. In statistical analysis, a model including the factors of dilution solution, time pm, and their interaction were run for the protein values, while the factors of dilution solution, material, time pm, and their interaction were run for the MFI values. The data were considered as repeated measures on time and analyzed using the PROC MIXED procedure of the SAS, where significant differences at 5% for least squares means were separated using the *Tukey-Kramer* test.

III. RESULTS AND DISCUSSION

A. Protein determination

There was an interaction ($P<0.05$) between dilution solutions and time pm for the protein values (Fig. 1). Meat samples taken at 7 and 14 days pm had higher ($P<0.05$) protein values than those ones taken at 1 day pm within the MFI buffer solution, while no significant differences were observed ($P>0.05$) across the times pm within the NaOH solution. A slight increase, but not significant, of the protein values across the times pm may be expected, because of the meat exudation during the aging [9,10]. Therefore, the protein values found for the suspense myofibrils diluted with NaOH solution seem to be closer to expected by not differing significantly as time pm increases. Mistakes in the protein values could result in wrong MFI values by loading more suspense myofibrils during final absorbance reading.

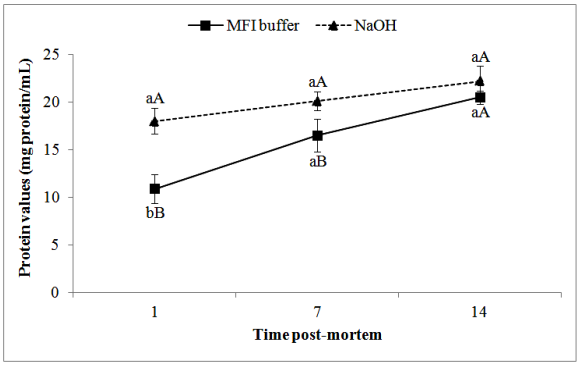

Figure 1. Protein values (mg protein/mL) in samples taken across the times post-mortem (pm) using different solutions for protein determination by biuret reaction. *Legend*: [a,b]Different lowercase letters across the times pm within the solutions differ significantly ($P<0.05$); [A,B]Different uppercase letters between dilution solutions within the times pm differ significantly ($P<0.05$).

Also, higher (*P<0.05*) protein values for NaOH solution than for MFI buffer solution were observed within the days 1 and 7 pm. This response pattern for the protein values did not occur (*P>0.05*) between the solutions within the day 14 pm. By the fact of the myofibrillar protein structures are firmer and less soluble in the early of the aging [11], it is possible that there is a substantial impact of the NaOH solution on the suspense myofibrils from days 1 and 7 pm.

B. MFI determination

The same interaction between dilution solutions and times pm found for the protein values was observed (*P<0.05*) for MFI values (Fig. 2). Meat samples taken at 7 and 14 days pm had higher (*P<0.05*) MFI values than those ones taken at 1 day pm within the NaOH solution, while no significant differences were observed (*P>0.05*) across the times pm within the MFI buffer solution. Here, a significant increase of the MFI values across the times pm would be expected, because a myofibrillar proteolysis occurs during the aging of the meat [12,13]. Hence, the unexpected result for MFI values obtained after protein determination using MFI buffer solution may be explained by the protein values found in those samples. A higher load of suspense myofibrils from meat samples taken at day 1 pm to make a 0.5 mg protein/mL solution may have corroborated for the lack of the effect of times pm within the MFI buffer solution.

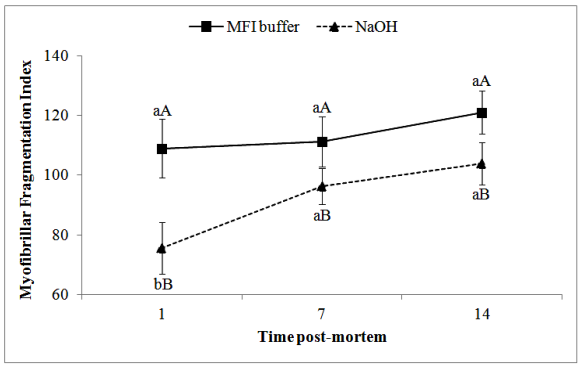

Figure 2. Myofibrillar Fragmentation Index values in samples taken across the times post-mortem (pm) using different solutions for protein determination. *Legend*: [a,b]Different lowercase letters across the times pm within the dilution solutions differ significantly (*P<0.05*); [A,B]Different uppercase letters between solutions within the times pm differ significantly (*P<0.05*).

Within all the times pm, the MFI values were higher (*P* < 0.05) for the MFI buffer solution than for the NaOH solution. A lower load of suspense myofibrils due to the use of right protein values (originated from NaOH solution) to make a 0.5 mg protein/mL solution may explain the lower MFI values.

A main effect (*P<0.05*) of materials used for the absorbance readings on the MFI values was found (Table 1). The MFI values from the cuvette and glass tube were similar (*P>0.05*), indicating that both the materials can be used without affect the MFI values. In this case, the use of the glass tubes would result in faster results with the same reliable of the results obtained from the cuvette (upper quality material). In both materials, the path traversed by the visible light bundles is a diameter of 10 mm.

The lowest MFI values (*P<0.05*) from the microplate point to an unreliable measurement, even though it is fast. In either cuvette or glass tube, the visible light bundles traverse a horizontal path crossing the suspense myofibrils. On the other hand, in microplate, the visible light bundles traverse a vertical path crossing the suspense myofibrils. Also, the path traversed by the visible light bundles is a diameter of 8 mm in the wells of the microplate. In this scenario, a lower absorbance value is found.

Table 1 Myofibrillar Fragmentation Index (MFI) values obtained by using different labware

Material	MFI values
Cuvette	127.3 (10.05)[a]
Glass tube	129.3 (7.45)[a]
Microplate	51.9 (5.04)[b]

Legend: [a,b]Different letters among the materials differ significantly (*P<0.05*).

IV. CONCLUSION

The protein values may be underestimated in meat samples taken at the first days of aging when the MFI buffer solution is used for the dilution of the suspense myofibrils. Because this, the MFI values may be overestimated due to a higher load of suspense myofibrils at the moment of the absorbance readings. The glass tubes may be used to optimize the MFI analysis, resulting reliable

values. Microplate should not be used to determine MFI values.

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