Rapid glycolysis test for detection of DFD meat

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Introduction

As it is known, DFD meat is considered to be relatively unsuitable for bacon type product and prepacked raw meat /Wirth, 1976., Bem et. al. 1976./ because of poor color stability and risk of short spoilage. Early post mortem detection of DFD muscles would be desirable in the slaughter line as additional parameter of carcass quality. Practically, PSE pork meat - exspecially extremely PSE ones - can be detected by measuring pH_1, while DFD meat is generally detected by measuring the pH on the day after slaughter /ultimate pH/. Honikel /1976/ and Honikel and Fischer /1977a, b/ have proposed to determine the R-value /inosine/adenine ratio/ combined with pH_1 to classification of normal, PSE and DFD pork meat at 1 hr post mortem. Since Ca and Mg ions regulate numerous biochemical reactions of muscle cell - Ca plays a decisive role in myofibrillar ATP-ase, contraction and also in the glycolytic process - it was assumed in this work, that a rapid pH decline would occur upon addition of Ca and Mg ions to the prerigor muscle sample provided that glycogen was present. When glycogen is absent or reduced, only a slight pH decline would be expected.

Material and Methods

Muscle samples: M. adductor was removed from pig carcass at 3o minutes post mortem. The muscle was cut into blocks of approximately 3 g. Blocks were randomized and two of them were used for each treatments Remaining blocks were packed into foil and stored at 4° C until determination of pH ultimate. For serial tests slices of approximately 3o g were removed from m. adductor at 3o minutes post mortem. Samples of approximately 3 g were taken from slices and placed into centrifuge tubes of 2o ml. Treatment was started not later than 5o minutes post mortem. Ultimate pH value was determined in the inner part of remaining slices after storage for 24 hr at 4° C.

Homogenisation: Immediately after addition of solution containing Ca and/or Mg at various concentration homogenisation was started /Ultra Turrax homogeniser/. No cooling was applied during homogenisation. Homogenisation was carried out with half speed /approximately 10000 rpm for 5 sec as moderate degree of homogenisation/ and also with full speed /approximately 10000 rpm for 5 sec followed by 20000 rpm for 5 sec as strong homogenisation/.

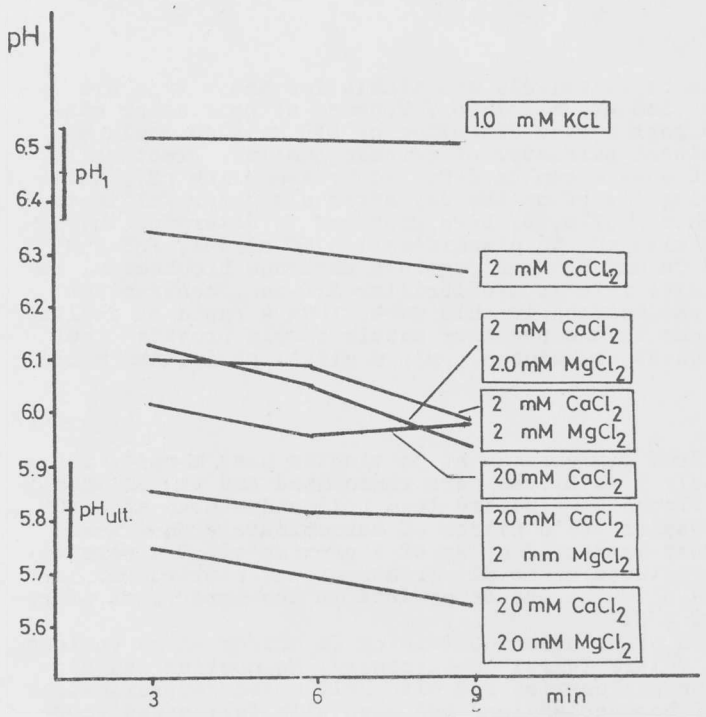
For pH determination Radelkis OP-109 pH-meter was used with combined glass electrode.

Results and discussion

In order to find the concentration of Ca and Mg ions which effectively stimulates glycolysis 3 ml of solutions were added to muscle samples taken from the same m. adductor /pH_1= 6,45±0,08/. Strong homogenisation was applied. The pH of homogenates were measured at 3, 6, 9 minutes after addition of solutions. Concentrations were as follows: 2 mM $CaCl_2$, 2 mM $CaCl_2$+2 mM $MgCl_2$, 2 mM $CaCl_2$+2o mM $MgCl_2$, 2o mM $CaCl_2$, 2o mM $CaCl_2$+2 mM $MgCl_2$, 2o mM $CaCl_2$+2o mM $MgCl_2$. Each solution contained also 1oo mM KCl. The latter was used to ensure ionic strenght of the medium. As Fig. 1. shows, ultimate pH of m. adductor /measured after 24 hr/ was approached in the homogenate only with solutions, which contained Ca ions at a concentration as much as 2o mM. 2o mM $CaCl_2$+2o mM $MgCl_2$ caused the most rapid pH fall. In the presence of KCl alone pH remained unchanged during this periode. In Fig. 2. the effects of 2o mM $CaCl_2$ alone and combined with 2 mM $MgCl_2$ and 2o mM $MgCl_2$ were compared as they influenced the pH fall of different individual muscles /3 ml solution and strong homogenisation was applied/. Analysis of variance showed significant effect of various solutions /$P < o,o1$/ and significant difference was found between individual muscles as they were influenced by various solutions /$P < o,o1$/. When 2o mM $CaCl_2$ was added, pH of homogenate was not reduced to the ultimate level /muscle 1 and 4/. Also, higher pH than ultimate was observed with 2o mM $CaCl_2$+2 mM $MgCl_2$ in the case of muscle 2 and 3 when pH of homogenate was measured after 3 minutes. In the case of 2o mM $CaCl_2$+2o mM $MgCl_2$ pH values of homogenates consistently approaches the ultimate pH of samples /muscle 1, 2, and 4/ or reduced to somewhat lower level /muscle 3/. Therefore, this concentration of $CaCl_2$ and $MgCl_2$ was chosen for the further investigations. In Fig. 3. effects of the amount of added solution /2o mM $CaCl_2$+2o mM $MgCl_2$+1oo mM KCl/ and the degree of homogenisation was shown with three different individual m. adductor. 3 and 6 ml solution was added /1:1 and 1:2 meat/solution ratio; strong and moderate homogenisation/. Analysis of variance showed significant effect /$P < o,o1$/ of homogenisation on the pH of homogenate measured at 3 and 6 minutes after addition of solution. The difference between strong and moderate homogenisation is particularly obvious with muscle 3 /o,25 pH/ - when moderate homogenisation was applied, pH of homogenate did not reach the ultimate pH whithin 6 minutes. Meat/solution ratio significantly influenced the pH of homogenate /$P < o,o5$/, but differences /in case of strong homogenisation/did not exceed o,o8 pH /muscle 1 and 2 measured at 6 minutes after addition of solution/, while with muscle 3 only a slight difference was found. Considering these results, 3 ml solution and strong homogenisation was applied whithout exact weighing of meat samples, when serial tests were carried out.

pH fall patterns of various m. adductors show, that pH values measured in the homogenate

Fig. 1. Effect of added Ca^{2+} and Mg^{2+} concentrations upon pH fall of m. adductor samples. Results are given as average of duplicate samples.



at 6 minutes after addition of solution can be found near the final pH values of homogenates. Only a slight or no reduction can be observed thereafter. It can be concluded from this result, that glycolysis appeared to be finished within a short time. Since bivalent cations cause contraction and other structural changes of muscle proteins /Hamm, 1972/ which alter their buffer capacity, pH of homogenate does not necesserily agrees with the ultimate pH of muscle. Weak acidity of $CaCl_2$ and $MgCl_2$ /pH 5.5/ also contributes to the reduction of pH in the homogenate.
In order to study the relationship between the pH measured in the homogenate and ultimate pH, nine different populations were tested /16-39 carcass/group/. Groups of carcasses /lean and fat pigs/ were randomly selected in the slaughter line. Each group consisted of normal and DFD samples. pH of homogenates /taken from m. adductor/ measured at 3 and 6 minutes after addition of solution were compared to the

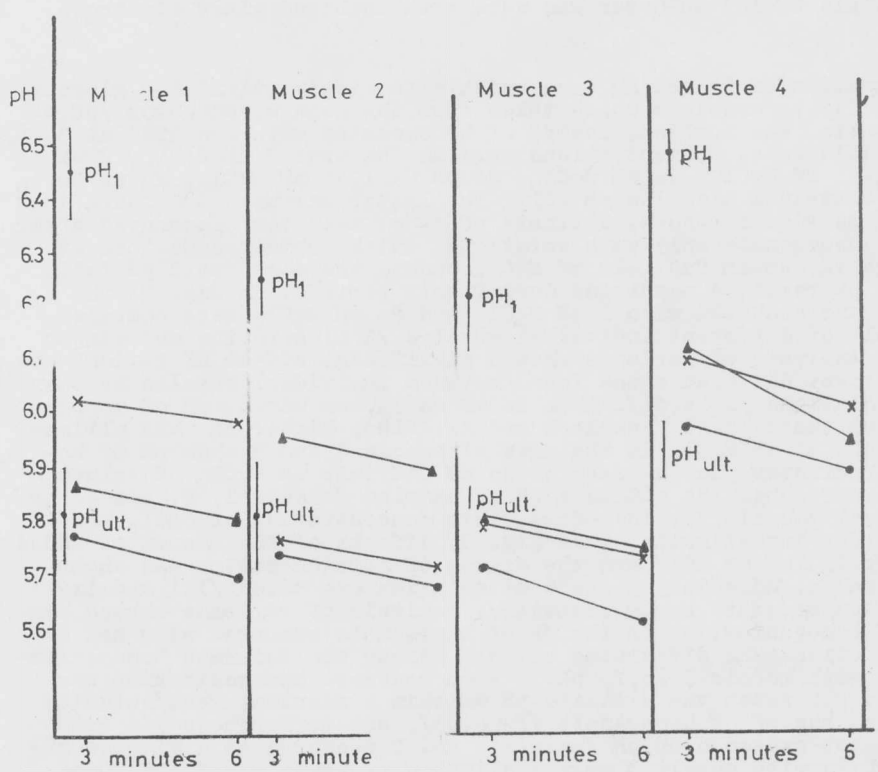


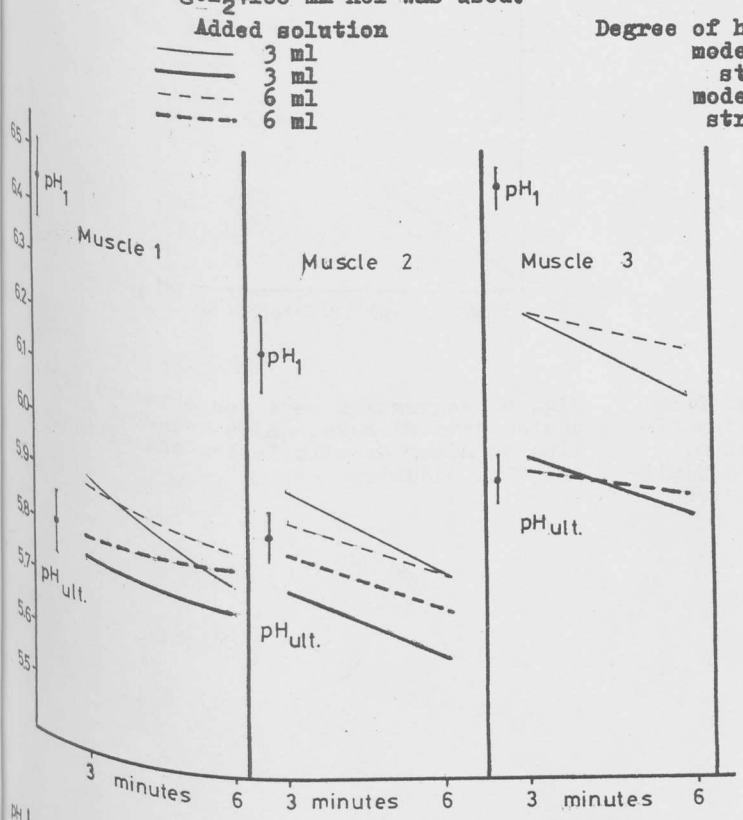
Fig. 2. Effect of 2o mM Ca^{2+} combined with Mg^{2+} upon pH fall of samples taken from different m. adductor.

× 2o mM $CaCl_2$
loo mM KCl

▲ 2o mM $CaCl_2$
2o mM $MgCl_2$
loo mM KCl

• 2o mM $CaCl_2$
2o mM $MgCl_2$
loo mM KCl

Fig. 3. Effect of amount of added solution /meat/solution ratio/ and degree of homogenisation upon pH fall of samples taken from different m. adductor. 2o mM $CaCl_2$ + 2o mM $MgCl_2$+1oo mM KCl was used.



ultimate pH. Nine linear regression equation were calculated. It was established, that regression lines appeared to be consistent when pH of homogenate measured at 6 minutes after addition of solution was considered /Fig. 5./. In the Fig. 6. regression equation is given calculated from pooled data of the nine groups./n=22o/. The standard error of estimate is relatively low, /o,o9 pH/ contrary to that of regression calculated from ultimate pH values and pH of homogenates measured at 3 minutes /y=o,66x+1,96 s_{xy}=o,17 pH/ r=o,8o/. The lower standard error of estimate in the former case reflects the more stationary stage of pH fall at 6 minutes after Ca-stimulation. This is in accordance with pH fall patterns in Fig. 4.

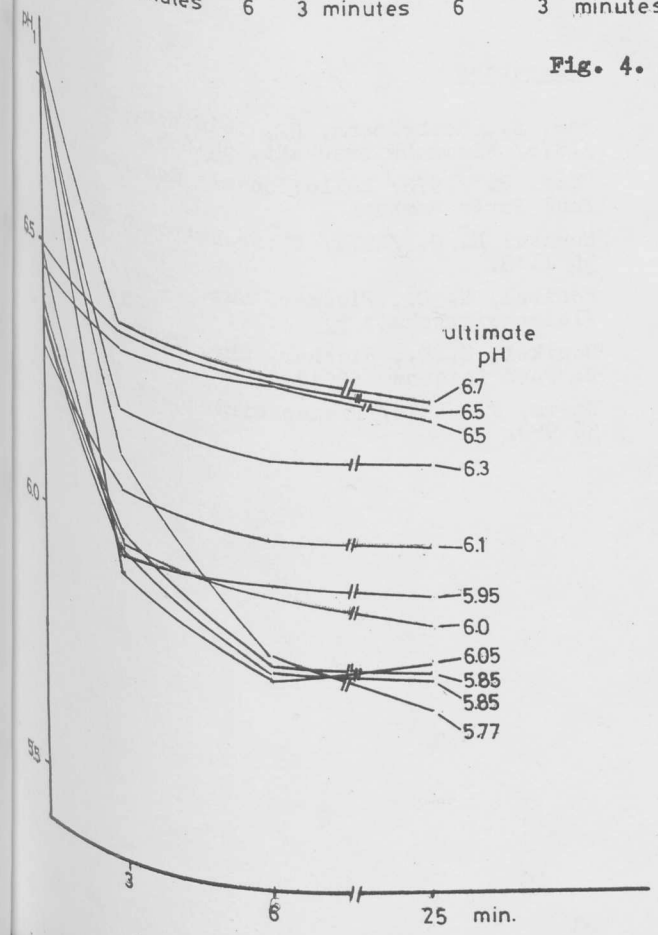


Fig. 4. pH fall in muscle homogenates of different m. adductor. 2o mM $CaCl_2$+2o mM $MgCl_2$ +1oo mM KCl was used.

In order to study the influence of post mortem time when Ca-stimulation started, treatments were carried out at 5o minutes and also at 2 hrs post mortem. In the latter case the pH of homogenates appeared to be lower /NS/. /Fig. 7./ Presumably, actual ATP concentration might influence the pH fall of the homogenate.

Conclusion

An increased rate of glycolysis was attained by addition of Ca and Mg ions to the porcine m. adductor samples. Owing to the standardisation of sampling time, homogenisation, meat/solution ratio and the time of pH determination in the homogenate, a close correlation was obtained between the pH of muscle homogenate and ultimate pH of muscle /r=o,88/. Using this relationship, ultimate pH of m. adductor can be estimated at an early post mortem time.

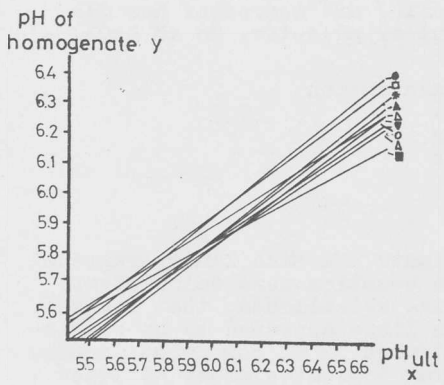


Fig. 5. Regression equations obtained from pH of homogenates measured at 6 minutes after Ca-stimulation and ultimate pH of m. adductor. Samples were collected from 9 different groups of carcasses at 3o minutes post mortem.

△ y=o,57x+2,41
▽ y=o,49x+2,91
□ y=o,66x+1,87
● y=o,63x+2,13
■ y=o,44x+3,19
▲ y=o,59x+2,31
o y=o,57x+2,42
▼ y=o,5ox+2,88
* y=o,57x+2,42

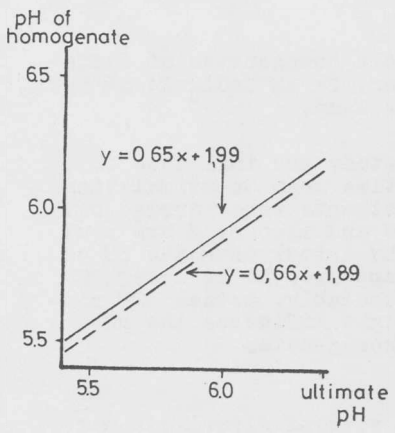


Fig. 7. Effect of post mortem time on the relationship between pH of homogenate measured at 6 minutes after Ca-stimulation and ultimate pH of m. adductor.

——— addition of solution at 5o minutes post mortem

- - - - addition of solution at 2 hour post mortem

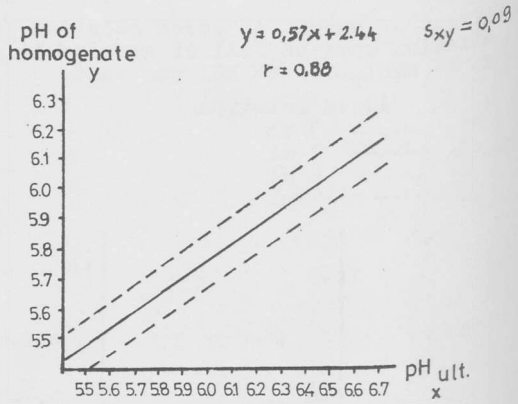


Fig. 6. Regression equation obtained from pooled data of homogenates measured at 6 minutes after Ca-stimulation and ultimate pH of m. adductor. n = 220.

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