A RAPID METHOD FOR THE DETECTION OF PSE- AND DFD-MEAT

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Extensive studies on porcine and bovine muscle showed that within a few minutes after death PSE- and DFD-muscles can be differentiated from normal muscles because of their lack of energy -rich substances like glycogen and ATP. Whereas PSE- and DFD muscles contain only low amounts of ATP, high concentrations of IMP and inosine are observed, which are formed by deamination of adenine nucleotides. In contrast normal muscles contain high levels of ATP, but low concentrations of IMP and inosine.

ATP like all the other adenine nucleotides exhibits a maximum of absorbance at 260 nm; IMP, inosine and hypoxanthine show their maximum of absorbance at 248.5 nm. The ratio (R) of the absorbances at 250 and 260 nm are commonly used as an index for purity in commercial available adenine and inosine nucleotide preparations. This ratio amounts to 0.78 - 0.80 for adenine nucleotides, ratios of 1.68 and 1.70 are measured for IMP and inosine. Therefore in normal meat soon after slaughter the R value should be low, whereas in PSE- and DFD-meat high R values can be expected. A considerable amount of investigations on different porcine and bovine muscles confirmed these considerations. Therefore it is possible to distinguish between normal meat on one hand and PSE- and DFD-meat on the other hand on the basis of its R value.

Muscles with PSE-conditions show a pH_1 value below 6.1, DFD muscles pH_1 values above 6.1. With these criteria in mind measuring the pH_1 and R value at the same time enables us to distinguish between normal, PSE- and DFD-meat. A rapid method will be described which allows to detect reliably normal, PSE and DFD- meat within 3-4 minutes.

METHODE RAPIDE POUR LA RECONNAISSANCE DE VIANDE PSE ET DFD

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De nombreuses études effectuées sur des muscles de boeuf et de porc ont montré que la viande DFD et PSE se différenciait de la viande normale par une déficience en composés énergétiques tels que le glycogène et l'ATP. Les viandes DFD et PSE se caractérisent par un faible taux d'ATP peu de temps après la mort et par une concentration élevée en IMP et inosine, produits de dégradation provenant de la désamination des adeninenucléotides. Dans le muscle normal, la concentration en ATP est par-contre élevée pendant les premières heures post mortem et la concentration en IMP est faible.

L'ATP a comme tous les adeninenucléotides un maximum d'absorption dans l'UV et ce à 260 nm; l'IMP, l'inosine et l'hypoxanthine ont eux leur maximum à 248,5 nm. Comme mesure de la pureté des adénine-nucléotides et des composés inosiniques nous avons retenu le rapport spectral (R) de l'absorption à 250 et 260 nm. Ce rapport est de 0,78 - 0,80 pour les adéninenucléotides, de 1,68 - 1,70 pour l'IMP et l'inosine. De ce fait il faut s'attendre à trouver une faible valeur de R dans une viande normale chaude contenant beaucoup d'ATP et s'attendre inversement à une trouver une valeur élevée de R pour les viandes PSE et DFD. Un grand nombre d'examens effectués sur divers muscles de porc et de boeuf environ 60 minutes après la mort confirme ces considérations. On peut donc différencier sur la base du rapport 250/260 le muscle normal du muscle DFD-PSE.

Les Muscles à tendance PSE ont une valeur de $pH < 6,1$, les muscles DFD ont une valeur de pH supérieure à 6,1. A l'aide de la valeur pH et du rapport R, on peut facilement faire la différence entre une viande normale, une viande DFD et une viande PSE. La méthode rapide décrite permet en l'espace de 3 à 4 minutes de faire sur le muscle juste après l'abattage la différence entre une viande normale, une viande PSE et une viande DFD.

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SCHNELLMETHODE ZUR ERKENNUNG VON PSE- UND DFD FLEISCH

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Umfangreiche Untersuchungen an Schweine- und Rindermuskeln zeigten, daß sich PSE- und DFD-Fleisch durch einen Mangel an energiereichen Verbindungen wie Glykogen und ATP vom Normalfleisch unterscheiden. Während der ATP-Gehalt im PSE- und DFD-Fleisch schon kurz nach dem Tode des Tieres niedrig ist, ist die Konzentration der durch Desaminierung der Adeninnucleotide entstandenen Abbauprodukte IMP und Inosin hoch. Im normalen Muskel hingegen ist die Konzentration an ATP in den ersten Stunden p.m. hoch, die IMP-Konzentration hingegen niedrig.

ATP weist wie alle Adeninnucleotide im UV-Bereich ein Absorptionsmaximum bei 260 nm auf; IMP, Inosin und Hypoxanthin jedoch haben ein Maximum bei 248,5 nm. Als Maß für die Reinheit von Adeninnucleotiden wie Inosinverbindungen wird das spektrale Verhältnis (R) der Absorption bei 250 und 260 nm angegeben. Dieses beträgt bei Adeninnucleotiden 0,78 - 0,80, bei IMP und Inosin 1,68 - 1,70. Daher ist in schlachtwarmem, normalem Muskel bei hoher ATP Konzentration ein niedriger Wert von R zu erwarten, in PSE- und DFD-Fleisch hingegen ein hoher Wert für R. Eine große Zahl von Untersuchungen an verschiedenen Schweine- und Rindermuskeln etwa 60 min p.m. bestätigen diese Überlegungen. Somit ist normaler Muskel von PSE- und DFD-Muskel auf Grund des 250/260 Verhältnisses zu unterscheiden.

Muskeln mit PSE-Eigenschaften haben einen pH_1-Wert $<6,1$, DFD-Muskeln haben einen pH_1-Wert $> 6,1$. Mit Hilfe des pH-Wertes und des Verhältnisses R kann man daher zuverlässig zwischen Normal-, PSE- und DFD-Fleisch unterscheiden. Es wird eine Schnellmethode beschrieben, die es gestattet, am schlachtfrischen Muskel innerhalb von 3 - 4 Minuten mit hoher Sicherheit zwischen normalem, PSE- und DFD-Fleisch zu unterscheiden.

СКОРОСТНОЙ МЕТОД ДЛЯ РАЗДЕЛЕНИЯ PSE и DFD МЯС

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Широкие иследования мускулов свинины и говядины показывают, что PSE и DFD мяса отличаются от нормального мяса отсутсвием богатых энергетических соединений, как гликоген и ATP. В то время, как содержание ATP в PSE и DFD мясе вскоре после убоя животного низко, концентрация продуктов, образованных дезаминацией аденинуклеотидов как IMP и инозина, высокая. В нормальных мускулях противположности, концентрация ATP в первых часах после убоя высокая, но концентраця IMP низкая.

ATP указывает в UV - части спектра максимум абсорбанци, при 260 nm , как и все другие аденинуклеотиды, IMP , инозин и гироксантин имеют при 248,5 nm . Мерой чистоты аденинуклеотидов и соединений инозина есть спектральная зависимость абсорбанци /R / при 250 по 260 nm . Эта зависимость заключается для аденинуклеотидов /0,78-0,80/ а для IMP и инозина /1,68-1,70 /. Потому надо ожидать низкого значения R в теплых нормальных мускулях после убоя: в PSE и DFD мясе напротив большого значения R . Большое число исследований на различных свиных и говажьих мускулях, около 60 после убоя потверждают эти ожидания. Таким способом можно различить нормальный мускул с PSE и DFD мускулов, но основе отношения 250/260.

Мускуля PSE качествами имеют pH > 6,1 DFD мускуля имеют pH<6,1 . С помощью оценки pH и соотношения R можно уверенно различить между нормальным, PSE и DFD мясом. Описывается скоростной метод позваляющий различить нормальное PSE и DFD мяса в свеже убитых мускулях в течение 3-4 мин с большой вероятностью.

RAPID METHOD FOR THE DETECTION OF PSE AND DFD MEAT

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INTRODUCTION

In the living muscle cell high-energy compounds such as creatine phosphate, adenosine triphosphate (ATP), adenosine diphosphate (ADP), hexose-phosphates and glycogen are present in well balanced concentrations. At death of the animal the blood circulation stops and the supply of the cell with energyrich substances and oxygen ceases. Also the removal of the metabolites is interrupted. Thus, the high energy compounds available at moment of death are used up in the continuing muscle metabolism, the rate of loss being dependent on several factors such as concentration of high-energy substances, genetic factors, stress and environmental conditions. The normal muscle during the first hours post mortem (p.m.) tries to maintain the well balanced ratio especially of adenine nucleotides. ATP is resynthetized by glycolysis and consequently the level of ATP remains constant or decreases slowly, whereas glycogen and hexose-phosphates decrease and lactate and protons are built up. The pH drops slowly from pH 7 to 5.5. Lateron after depletion of glycogen a loss of ATP occurs. ATP is dephosphorylated to ADP and adenosine monophosphate (AMP), the latter is deaminated quite instantaneously to inosine monophosphate (IMP). During further storage of muscle, IMP is enzymatically transformed to inosine and finally to hypoxanthine. In general, the postmortem breakdown of adenosine nucleotides to hypoxanthine derivatives takes about 12 to 24 hours in normal muscle. This change has been reported in the literature as a method for determining the state of rigor mortis (1,2).

Deviations from the normal time slope of postmortem changes can be observed quite often in porcine and sometimes also in bovine muscles. So, PSE muscle shows already 45 minutes p.m. very low levels of ATP and glycogen and low pH_1 values but elevated levels of IMP and lactate. After 24 hours p.m. the differences between PSE and normal meat are not significant with regard to those compounds and pH (table 1). On the other hand, in DFD muscles 45 min. p.m. low levels of ATP, glycogen and lactate are found combined with high pH_1 values. 24 hours p.m. the pH is higher and the level of lactate lower than in normal muscle (table 1). There exists a highly significant correlation between pH_1

table 1

Average Nucleotide and Derivatives Concentrations in porcine muscle at times p.m. (3)

nucleotide	concentrations µMol/g meat			
	normal		PSE	DFD
	1 hour	24 hours	1 hour	1 hour
ATP	3 - 5	0 - 0.2	0 - 0.60	0 - 1.05
ADP	1 - 2.5	0.8-1.5	0.96-1.10	0.70-1.40
AMP	0.5-1.0	0.2-0.5	0.04-0.30	0.13-0.45
IMP	1 - 3	6 - 8	4.40-7.10	2.30-5.10
inosine	0.5-1	1.5-3	0.35-1.15	1.15-2.00
hypoxanthine	0.2-0.3	0.5-1.0	0.10-0.20	0.10-0.45
A[a]	4.5-8.5	1.0-2.2	1.0-2.0	0.83-2.90
I[b]	1.7-4.3	8 - 12	4.85-8.45	3.55-7.55

a) sum of adenine nucleotides

b) sum of IMP, inosine and hypoxanthine

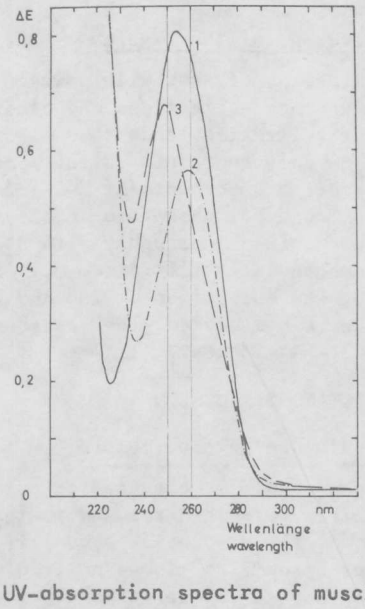

fig.1 UV-absorption spectra of muscle extracts with perchloric acid in porcine muscle 45 min. p.m. A Beckman spectrophotometer DB-G was used with phosphate-buffer as reference in 10 mm kuvettes.

Curve 1: 2.5×10^{-5} M ATP + 2.5×10^{-5} IMP in 0.1 M phosphate buffer pH 7.0. Curve 2: Extract with perchloric acid of normal pig muscle (M.long.dorsi) pH_1=6.3, R=0.84. Curve 3: Extract of PSE muscle from pig (M.long.dorsi) pH_1=5.55, R=1.30

and PSE conditions of pork (watery, pale), but not between pH_1 and DFD conditions of meat (dry, dark) because both muscle types, DFD and normal, show high pH_1 values. However, between the ATP and IMP level and pork quality (PSE and DFD) a highly significant correlation exists (3) because in both muscle types, PSE and DFD, the breakdown of ATP to IMP occurs much faster than in normal muscle. Consequently for objective quality evaluation a fast and simple method for the detection of this transformation would be highly desirable. But the usual chromatographic or enzymatic methods for the determination of ATP and/or IMP are too time consuming. However, the change in the UV absorbance occuring during the deamination of the adenine moiety p.m. can be used as an easy and reliable objective method for differentiation between normal meat and PSE or DFD meat.

All adenine nucleotides show an absorption maximum at 259 - 260 nm, IMP and inosine at 248.5 - 249 nm and hypoxanthine at 250 nm. The ratio of absorbance at 250 and 260 nm is used in commercial purine preparations as a reference for purity. This ratio amounts to 0.78 - 0.80 for adenine nucleotides, to 1.68 - 1.70 for IMP and inosine and to 1.32 for hypoxanthine. In normal prerigor muscle with its high content of adenine nucleotides the 250/260 ratio (called R value furtheron) should be low, in PSE and DFD meat it should be higher because of its high IMP, inosine and hypoxanthine concentrations (fig. 1). Therefore, a measurement of the R value in an extract of muscle tissue with perchlorid acid should give a reliable information on meat quality, because between 250 and 260 nm no other acid-soluble compounds of muscle tissue absorb to a noteworthy extent.

MATERIALS AND METHODS

Pig carcasses (German Landrace) of about 100 kg weight and both sexes were randomly selected, 52 samples of M. semimembranaceus and M. longissimus dorsi were removed between 40 and 45 min. p.m. The pH was measured immiately. The muscle samples were divided into 4 parts. One was frozen at once in a vacuum sealed bag at -20°C for the enzymatic assay of ATP (4), the second one was used for measuring the waterbinding capacity (WBC) according to Grau-Hamm (5) within the next 15 minutes. In the third part the R value was determined at the same time. Finally a sample was vacuum packed and stored for 24 hours at + 4°to + 8°C for the pH measurement at 24 hours p.m.

Cattle carcasses (Fleckvieh, weight 400 - 450 kg) of both sexes were randomly selected in the abattoir over several weeks and investigated in the same way as reported for pig carcasses. 62 samples of M.semimembranaceus, M. obturator internus, M. psoas major, M. sternomandibularis and diaphragm obtained within 45 min. to 1 hour p.m. were studied.

Preparation of the samples

By extraction of meat with perchloric acid in a homogenizer proteins and nucleic acids are precipitated whereas nucleotides and other low molecular compounds are dissolved. The following centrifugation or filtration leads to a clear solution which contains besides nucleotides, inosine and hypoxanthine only inorganic salts, some vitamins, peptides and amino acids. These latter compounds show little or no absorbance at 250 - 260 nm, as illustrated in fig. 1 (compare the shape of curve 1 with curves 2 and 3); therefore, nucleotides can be detected quite easy without interference. 1 - 3 g meat are homogenized thoroughly with 5 - 10 ml 1M perchloric acid in a "Bühler Homogenisator" for 30 sec. The homogenate is filtrated, 0.1 ml of the filtrate is diluted into 4.9 ml 0.1M phosphate buffer pH 7.0. The absorption at 250 and 260 nm is measured with phosphate buffer as reference and the R value is calculated. The procedure from receiving the meat until the evaluation of the result takes 3 - 4 minutes.

RESULTS AND DISCUSSION

1) Studies on porcine muscle

pH_1, pH_{24} and WBC were used as parameters for differentiation between normal, PSE and DFD meat. These parameters were then related to the ATP concentration and the ratio R in order to evaluate the reliability of the R value as a quality index (fig.2). For normal meat pH_1 was above 5.9, pH_{24} below 5.75, WBC was below 5 cm^2 loose water/0.3 g muscle; in PSE muscles pH_1 was below 5.9, pH_{24} below 5.6, WBC above 5 cm^2 loose water/0.3 g of muscle; in DFD muscles pH_1 was above 5.95, pH_{24} no more than 0.1 pH units different from pH_1 and above 5.85.

Fig. 2 below shows that all samples of normal meat had an ATP concentration above 1 µMol ATP/g and except 1 sample pH above 5.9. All PSE muscles were below pH 5.9 except 1 and below 1 µMol ATP/g meat. DFD muscles showed values above 5.95 and ATP concentrations below 1.2 µMol ATP/g. Plotting the R value against the pH (fig. 2 upper part) separated normal, PSE and DFD meat very well. All normal muscle samples exhibited R values below 1.05, all PSE and DFD samples R values above 1.10. PSE and DFD muscles are well separated by their pH_1 value.

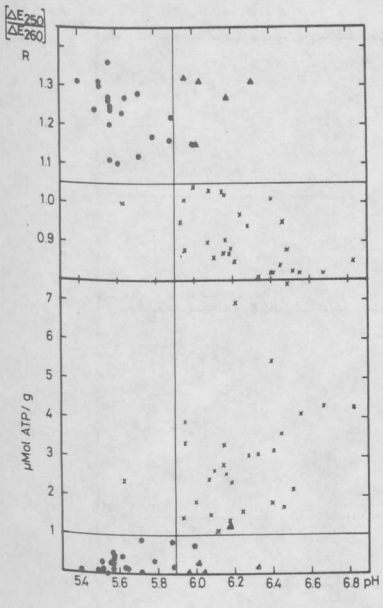

fig. 2: Relation between ATP concentration, pH_1, R value and meat quality in porcine muscle.

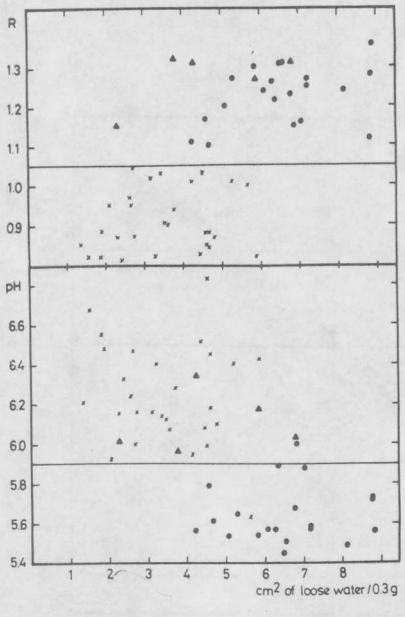

fig. 3: Relation between R, pH_1 and WBC and meat quality in porcine muscle.

52 muscle samples from pig carcasses removed 45 min. p.m. The muscles studied are reported in the text. The limits of ATP concentration, R, pH_1 of the three meat qualities are drawn in the figures. (x) normal-, (●) PSE-, (▲) DFD-meat.

Fig. 3 below shows the relation of the pH_1 values and the WBC. Whereas the pH_1 separates PSE on the one side and DFD and normal on the other side, the WBC of all three muscles conditions interfere somewhat with each other. The same is true for plotting R with the WBC (fig. 3 upper part). The R value separates normal from PSE and DFD, but DFD and PSE samples interfere with each other. Therefore only pH_1 and ATP concentration or R value allow a clear differentiation of the three quality classes of meat. The evaluation of the ATP concentration, however is time consuming while measuring the R value is easy and fast.

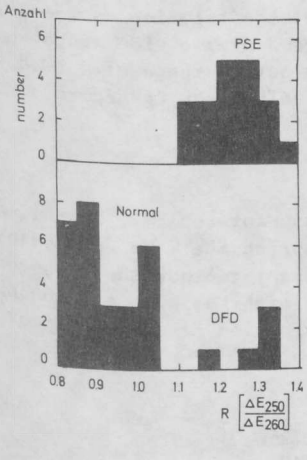

Fig. 4: Frequency of normal, PSE and DFD muscles from pig carcasses plotted vs. R. The muscles studied are reported in the text.

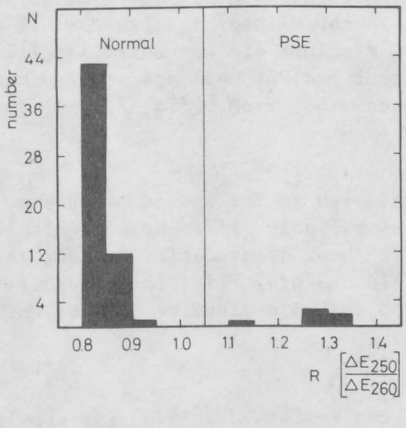

Fig. 5: Frequency of normal and PSE bovine muscles plotted vs. R. The muscles studied are reported in the text.

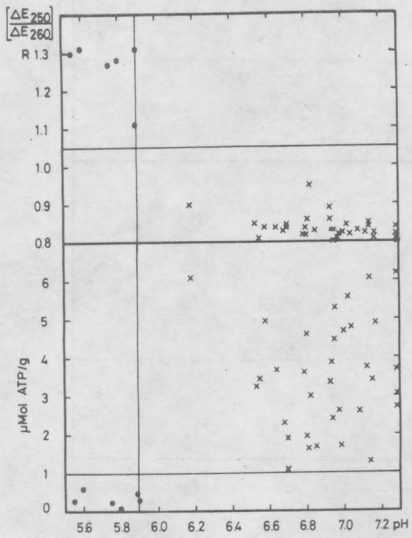

Fig. 6: Relation of ATP concentration, pH_1, R values and meat quality in bovine muscle.

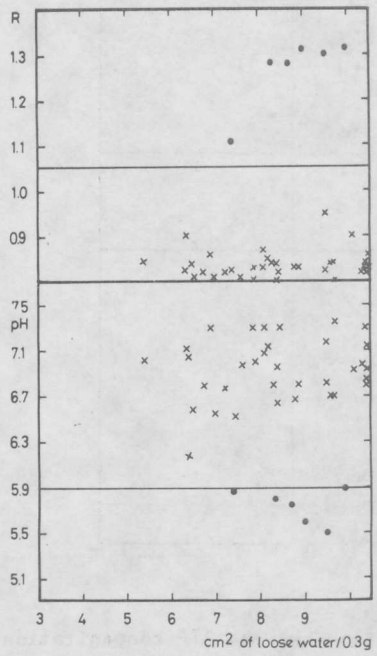

Fig. 7: Relation of R, pH_1, WBC and meat quality in bovine muscle.

Muscle samples from cattle carcasses were removed 45 min. to 1 hour after death. The muscles studied are reported in the text. The limits of ATP concentrations, R values and pH_1 of the normal and PSE muscles are drawn in the figures for better understanding. (x) normal, (●) PSE meat. 52 muscle samples have been studied, 27 of them were normal with a R value below 1.05 (fig. 4), the main part of them being below 0.90. 20 samples exhibited PSE conditions with R values above 1.10. 5 DFD muscles were found. All R values were above 1.15.

2) Studies on bovine muscles

56 samples (fig. 5) of the 62 muscles studied exhibited normal meat conditions, 6 muscles, exclusively samples from M. psoas major, showed PSE characteristics. Unfortunately, no DFD muscles occured in the material studied as yet. All normal samples showed R values in the small range between 0.8 and 0.95. PSE muscles were all above 1.10. In Fig. 6 we represent the relation between ATP, pH_1 and R value. In normal beef muscles the ATP concentration is spread over a wide range while the corresponding R values are concentrated (fig. 6 upper part) in a narrow range of R (0.8 - 0.9). The values of normal and PSE meat are well separated, while the WBC values can be separated only by additional measuring of pH_1 (fig. 7)

CONCLUSION

150 muscles studied so far showed no runaway between R values and meat quality. Normal, PSE and DFD muscles can be reliably differentiated within 3 - 4 min. by measuring the R value together with the pH in the first hour after death. In meat research this method might replace the time consuming determination of ATP and/or IMP for screening tests. But the procedure might be also of economical interest because the objective evaluation of meat quality allows the elimination of abnormal meat before processing.

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