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PROTEINS OF MEAT AS A POTENTIAL INDICATOR OF ITS QUALITY – A REVIEW*

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There are different ways to define meat quality and various compounds may be employed as indicators of its quality. Progress observed in biological sciences, especially in proteomics, indicates that proteins building meat structures as well as those taking part in metabolic processes, by being a constituent part of enzymes, can provide indicators of meat quality. Meat quality, from the practical point of view, plays a particularly important role in the case of meat intended for culinary purposes.

This study pays special attention to protein transformations associated with the most important meat properties, *i.e.* colour, water holding capacity/juiciness and tenderness. It was indicated how, using tools of genomics and proteomics, it is possible to observe differences associated with quality. Special attention was paid to interconnections between oxidation processes and changes in meat colour and lipids but also protein transformations, including drip development.

INTRODUCTION

Meat quality can be expressed by determining a number of its properties of which, for the consumer and manufacturer of meat products, the most important ones include: colour, juiciness, taste, smell and texture. The last of the above-mentioned properties can be assessed in different ways, although in the case of meat, attention is paid, in particular, to tenderness.

There are many factors influencing the quality of meat raw material [Litwińczuk *et al.,* 2004] of which the genetic factor is considered to be of key importance. Once the desirable level of advantageous quality traits has been reached by means of genetic 'manipulations', other factors begin to play a leading role. They include factors associated with production conditions (feeding and environment of animals) as well as those involved in the initial processing of slaughter animals such as animal transport and slaughter and the process of carcass or meat chilling. These are the first conservation treatments whose aim is to preserve the positive properties of the slaughter raw material for the longest possible time. The stage of the initial meat processing may preserve the advantageous meat properties but it can also worsen them. The above remarks are associated with the impact of these factors on animal metabolism and, hence, on changes which occur in the muscles after slaughter and affect meat quality. In this regard, proteins play a particularly important role because they are building blocks of muscle cellular structures and, in addition, they constitute components of enzymes. Therefore, they play both dynamic and static functions. However, the above diversification does not mean that the muscle structures and tissues surrounding them making up the slaughter raw material do not undergo any changes. They change with the age of animals. These changes are particularly rapid during the period of growing but, once the animals achieve maturity, these changes slow down. Most animals that are slaughtered in order to obtain raw material for culinary purposes are young and their cellular structures are already well developed and the metabolic activity of their organisms is high. When looking for meat quality indicators, researchers often wonder which compounds can best be used to represent them. Since proteins make up a considerable part of the meat basic composition and their changes are associated with its quality, it is not surprising that it is these compounds that are frequently looked to as a source of possible indicators/markers of meat quality. Proteomics, as the science of proteins equipped with a number of new methods and research tools, allows following these changes.

PROTEINS AND MEAT COLOUR

Myoglobin and hemoglobin are both proteins and they can have a decisive influence on meat colour. The latter of the two compounds constitutes the basic blood dye and can, primarily as a result of improper bleeding during the slaughter, remain in meat and affect its colour. The correct meat colour (pink or light-red) is associated mainly with the occurrence of myoglobin (deoxymyoglobin) or its derivative – oxymyoglobin [Livingston & Brown, 1982]. All after-slaughter treatments aim at preserving these two forms of myoglobin for as long as it is possible. That is why, when meat is packed using modified atmosphere it often contains oxygen (usual-

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ly more than 20%) and until recently, mixtures were applied which contained even up to 80% oxygen [Jayasingh *et al.,* 2002]. Nowadays, such high levels of this gas are avoided for the simple reason that the oxidation processes of the meat fat fraction affecting also its quality, progress very rapidly and meat undergoes spoilage as indicated by its colour. The above-mentioned treatment, although it does protect the meat colour, also accelerates meat deterioration and misleads consumers as to meat quality [Juncher *et al.,* 2001]. Moreover, it was noticed that meat stored in the atmosphere containing very high quantities of oxygen was tough. Investigations undertaken to elucidate this phenomenon revealed that oxidation processes may reduce meat tenderisation processes as it turned out that protein oxidation can inhibit significantly the activity of μ-calpains [Guttmann *et al,.* 1997; 1998; Rowe *et al.,* 2004]. The above remarks explain partially the problem of the worse tenderness of the PSE meat whose colour directly after slaughter is very light and its spectra indicate the presence of oxy-myoglobin which is not usually found in the case of meat of normal quality. The appearance of oxy-myoglobin in the meat of pigs subjected to stress may result from the increase of temperature in the muscle, which leads to increased protein denaturation also associated with an increased demand for oxygen [Rosenvold & Andersen, 2003]. This phenomenon is observed especially in muscles usually recognized as darker, *e.g. m. biceps femoris* and *m. semitendinosus*. This is due to the inactivation of the met-myoglobin reductase. As a result of this reaction, the colour stability of meat during storage can be restricted. Stress susceptibility makes differences between muscles more visible. Mutation in the RYR1 gene usually leads to increased lightness [Kuchenmeister *et al.,* 2000]. However, it is possible to prevent the process of oxidation by applying antioxidants in animal nutrition [Rowe *et al.,* 2004]. It is also well known that vitamin D_3 added to diets prevents the occurrence of wateriness [Wilborn *et al.,* 2004].

In the case of the influence of the acid meat gene, muscles of the rn^+/rn^+ genotype are redder, especially with regard to the RN^-/rn^+ genotype [Moeller *et al.,* 2003]. Lindahl *et al.* [2004] claims that differences in the red and yellow shades of pork meat between pigs of different alleles at the PRKAG3 locus are associated with the oxido-reductive condition of the myoglobin. Smaller colour changes can be observed in the meat of genotypes with higher pH, which is associated with the increased oxygen consumption accompanied by increased oxy-myoglobin stability and the activity of met-myoglobin reductase. It is worth emphasising here that the meat quality, including, in particular, that of *musculus longissimus dorsi*, is usually correlated with the magnitude of the glycolytic potential of muscles and the content of free glucose [Moeller *et al.,* 2003; Hamilton *et al.,* 2003; Juncher *et al.,* 2001].

Moreover, two-dimensional electrophoresis in combination with mass spectrometry of the protein sarcoplasm of *semitendinous* muscles varying with regard to their colour lightness indicates a correlation between the colour of muscles and the type of proteins (including those of mitochondrial character) contained in them [Morzel *et al.,* 2005]. Out of 17 proteins significantly diversifying light and dark muscles, the authors attention was caught by two proteins from the so-called accompanying proteins and antiquitin. The first two act as proteins protecting against denaturation, whereas antiquitin is associated with maintaining muscle osmotic pressure. It is believed that in future some of these proteins may serve as markers of meat quality.

It is quite clear from literature on the subject that animal movement may affect changes in muscle metabolism resulting in the increased proportion of myoglobin and enzymes of oxidative character [Jørgensen & Hyldgaard-Jensen, 1975]. The above contributes to improved meat quality. Therefore, it seems expedient for new breeding-production programs to take into consideration not only possibilities of genetic and nutritional impacts but also environmental factors, including movement of animals.

WATER HOLDING CAPACITY AND PROTEIN TRANSFORMATIONS

Water holding capacity is associated with various phenomena which, despite very similar result, need not necessarily be connected with transformations of the same proteins. Generally speaking, it is assumed that the process of meat proteolysis assists increased water holding capacity. Measurements of water holding capacity taken using traditional methods indicate increased water binding with the progress of meat maturity. The rate of this process varies and depends on many factors of which the most important ones include: animal species, their age, type of muscle and the rate of changes which take place in the muscle tissue after slaughter [Hamm, 1972].

As a rule, accelerated after-slaughter changes lead to a significant confinement of meat water holding capacity. Basically, the difference in the water binding strength between normal meat and watery meat remains unchanged throughout the storage period after slaughter. Also acid meat is characterised by low water holding capacity and if it finds its way into industry unrecognised, it causes considerable thermal drips. Recently, researchers have been focusing their attention on normal quality meat which is characterised by large free drip during its cold storage [Koćwin-Podsiadła & Krzęcio, 2005]. Some studies indicate genetic preconditioning of calpastatin gene (CAST) [Koćwin-Podsiadła *et al.,* 2003], although it is not always possible to confirm this dependence. Studies of Kurył *et al.* [2004] show that the above-mentioned phenomenon can be attributed to the modifying influence of the CAST genotype on changes taking place in the muscle tissue. It is probable that the cause of the occurrence can be explained by the reaction similar to the cold shortening caused by the increased secretion of calcium ions from the sarcoplasmic reticulum. Perhaps, in the case of pork, rapid chilling of carcasses leads to a similar reaction resulting in an increased drip which is also characteristic for the cold shortening.

The latest investigations point to still one more possibility which could elucidate the background of the variations in the size of the meat drip loss [Huff-Lonergan *et al.,* 2005]. The observed variations can be attributed to differences in the rate of proteolysis of proteins forming intermediate filaments such as talin, synemin, dystrophin and vinculin [Bee *et al,.* 2004; Kristensen & Purslow, 2001; Morrison *et al.,* 1998; Melody *et al.,* 2004]. Their reduced degradation can be associated with increased drip. The above proteins belong to cytoskeletal proteins and undergo proteolysis relatively quickly,

much faster than titin. It is suspected that if their proteolysis is slowed down, then they undergo the same contraction as the whole muscle. It is believed that the mechanism of this process is as follows: the intermediate filaments, by connecting myofibrils with one another as well as muscle fibres with the membrane surrounding bundles of muscle fibres, transfer tensions occurring during muscle contraction and press water out from spaces between them causing drip loss. The smaller the degradation of intermediate filaments, the stronger are the forces pressing the water out and the higher the drip.

Changes that occur after slaughter and which are associated with the progressing acidification, decrease of temperature in muscles during chilling, increase of ion strength and the increasingly smaller capability of the muscle to maintain reductivness of the environment can modify the above-described processes. However, they can lead not only to changes in the size of the drip but also to changes in the rate of the tenderisation process.

MEAT TENDERNESS AND PROTEIN CHANGES

A phenomenon typical of processing taking place after slaughter is muscle stiffening as a result of the process of myofibril contraction. Limiting the extent of this contraction or preventing it, improves meat tenderness. This meat property – influencing very strongly its quality – is affected, similarly to its water holding capacity, by variable factors. Experiments involving the evaluation of genetic impacts, metabolism of muscle fibres as well as the character and quantity of proteins influencing this meat trait are particularly interesting in this respect.

Until fairly recently, it was widely believed that "fine texture or graininess" of muscles (small diameter of muscle fibres) enhances its tenderness. However, experiments carried out on "double muscled" cattle, which have thick fibers, showed that the observed better meat tenderness of this type of animals resulted from nearly twice as low content of connective tissue proteins [Ngapo *et al.,* 2002]. This finding indicates that collagen – its quantity and quality – can affect the tenderness of meat obtained from both young and old animals. The above conclusion refers, primarily, to bovine meat [Christensen *et al.,* 2005] but a similar relationship is also found in the case of pork as variations in the size of muscle fibres between some breeds are sometimes very big [Vautila *et al.,* 2005]. An additional factor which is connected with the diameter of muscle fibres is their metabolism and this character is controlled, primarily, by myosin. A characteristic feature of thicker meat fibres is a smaller share of slowly contracting fibres, of oxydative nature. The fibers with bigger diameter contain less myoglobin in relation to slow twich, oxidative fibres which are dominant in red fibres, usually of small diameter. However, white fibres are fairly varied with regard to the rate of the relaxation process and this, as reported by Grześ *et al.,* [2005a], may influence the process of meat tenderisation. These interrelationships can be further modified both in the results of genetic as well as environmental factors [Jørgensen & Hyldgaard-Jensen, 1975; Mozdziak *et al.,* 1998]. The slaughter of animals at higher weight can also result in a change of muscle metabolism of pigs [Grześ *et al.,* 2005b] as well as changes in the rate of tenderisation processes and water binding by the meat tissue.

The course of the tenderisation process is particularly badly affected by the stress susceptibility gene RYR1. The largest of the cytoskeletal proteins – titin – undergoes the fastest degradation in normal quality muscles, whereas troponin T (Tn-T) – is degraded fastest in muscles with accelerated glycolysis characterised by wateriness (PSE) [Pospiech *et al.,* 2004; Mikołajczak, 2004]. In acid muscles, usually characterised by better tenderness, titin is degraded somewhat faster and the decomposition of Tn-T is somewhere between that observed in normal quality muscles (RFN) and PSE [Mikołajczak, 2004].

It is interesting to note that the degradation of the two above-mentioned proteins results in a significant increase of the degradation products with the isoelectric point (pI) above 7.7. In the case of myosin, proteolysis led to slight changes which concerned pI below 5.59 [Mikołajczak *et al.,* 2005b]. In the case of watery meat, the proportion of proteins of pI below 4.69 and ranging from 4.7–5.59 was correlated with the level of the centrifugal drip after one week of storage in cold store ($r=0.93$ and $r=0.96$ at $p<0.05$, respectively). In the same raw material, a high correlation was also found between the quantity of proteins of pI ranging from 5.6 to 7.69 and from 7.7 to 8.79 and losses during storage after 168 h ($r=0.94$ and 0.93 at $p<0.05$, respectively).

Moreover, observations of the course of meat tenderness changes indicate that this process may follow a fairly varied course. Iwańska *et al.* [2005] identified 4 groups of pork muscles: very tough (I) and very tender (II) throughout the entire 6-day storage period and tenderising muscles from large (III) or average (IV) values of shear forces. There were significant differences between individual muscle groups in the amount of titin and Tn-T or products of their degradation or aggregation observed on the separations of the examined muscles of the centrifugal drip obtained from them and this constitutes the basis for the elaboration of meat tenderness markers. Experiments associated with it employ a number of analytical techniques of which the most promising are: immunoblotting after two-dimensional electrophoresis or the combination of the two-dimensional electrophoresis (2D) with mass spectrometry. In the case of bovine meat, the Tn-T degradation product of 1735 Da weight [Mullen *et al.,* 2000] and lactate dehydrogenase [Sierra *et al.,* 2005] are suggested as the potential markers of tenderness. Both of them can also be taken into consideration as potential candidates for tenderness markers in the case of pork [Mikołajczak *et al.,* 2005a]. Some reports indicate the amount of free amino acids [Mullen *et al*., 2000] as an indicator of meat tenderness but the correlation between their concentration and the magnitude of the shear force as a rule ranges from $r = -0.35 \div 0.42$.

SUMMARY

Identification of specific proteins as markers of selected meat properties could be utilised for the testing of the final meat quality. The above-quoted data indicate that investigations in this area are fairly advanced, although intensive work is still continued to find rapid and simple tests of meat quality. Among the most interesting developments in this field, the following deserve to be mentioned: trials to utilise autofluorescent measurements and relatively long-running tests connected with the assessment of changes of electric proper-

ties of meat (conductivity, impedance) to determine and predict the culinary and processing properties of meat. It is difficult to envisage, at the moment, the application of markers in meat shops offering meat of declared quality. However, it is believed that they can be successfully employed to select animals for the production of culinary meat. In this case, they could assist both breeders and processing industry in their attempt to produce meat raw material of the highest quality standards.

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BIAŁKA MIĘSA JAKO POTENCJALNY WSKAŹNIK JEGO JAKOŚCI – ARTYKUŁ PRZEGLĄDOWY

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Jakość mięsa można definiować w różny sposób, różne też związki mogą służyć jako jej wskaźniki. Postęp, jaki dokonuje się w naukach biologicznych, w tym szczególnie w proteomice, wskazuje, że białka tworzące struktury mięsa, a także uczestniczące w procesach metabolizmu będąc częścią składową enzymów, mogą stanowić wskaźniki jakości mięsa. Z punktu widzenia praktycznego jakość mięsa odgrywa szczególną rolę w przypadku mięsa przeznaczonego na cele kulinarne.

W pracy zwrócono szczególną uwagę na przemiany białek związane z kształtowaniem najważniejszych cech mięsa, tj. barwy, wodochłonności/soczystości i kruchości. Wskazano jak wykorzystując narzędzia genomiki i proteomiki można zaobserwować różnice związane z jakością. Szczególną uwagę zwrócono na powiązanie procesów utleniania nie tylko ze zmianami barwy i lipidów mięsa, ale również z przemianami białek, w tym powstawaniem wycieku.