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Review

Biomarkers of meat tenderness: Present knowledge and perspectives in regards to our current understanding of the mechanisms involved

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ABSTRACT

Biomarkers of the meat quality are of prime importance for meat industry, which has to satisfy consumers' expectations and, for them, meat tenderness is and will remain the primary and most important quality attribute. The tenderization of meat starts immediately after animal death with the onset of apoptosis followed by a cooperative action of endogenous proteolytic systems. Before consideration of the biomarkers identified so far, we present here some new features on the apoptotic process. Among them, the most important is the recent discovery of a complex family of serpins capable to inhibit, in a pseudo-irreversible manner, caspases, the major enzymes responsible of cell dismantling during apoptosis. The biomarkers so far identified have been then sorted and grouped according to their common biological functions. All of them refer to a series of biological pathways including glycolytic and oxidative energy production, cell detoxification, protease inhibition and production of Heat Shock Proteins. Some unusual biomarkers are also presented: annexins, galectins and peroxiredoxins. On this basis, a detailed analysis of these metabolic pathways allowed us to identify some domains of interest for future investigations. It was thus emphasized that mitochondria, an important organelle in the production of energy from carbohydrates, lipids and proteins are a central element in the initiation and development of apoptosis. It was therefore stressed forward that, in fact, very little is known about the *postmortem* fate of these organelles and their multiple associated activities. Other topics discussed here would provide avenues for the future in the context of identifying reliable predictors of the ultimate meat tenderness.

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1. Introduction

Among the different qualities of meat, tenderness is and will always be the first quality sought by the consumer. Since decades, the mechanisms of meat tenderization have focused much interest from meat scientists. Despite these extensive efforts, these mechanisms are still unclear and this probably explains the fact that we have not yet identified a good marker of this quality (Kemp, Sensky, Bardsley, Buttery, & Parr, 2010).

Nevertheless, there is now a general agreement on two major questions: (1) the meat tenderizing mechanisms are enzymatic in nature and involve several intracellular proteolytic systems and (2) the first step of the conversion of muscle into meat is the onset of apoptosis, a finely regulated and complex energy dependent cell death process (Ouali et al., 2006). In this context, the first proteolytic system likely to be involved is the group of initiator caspases followed by executor caspases, responsible for the degradation of proteinaceous cell constituents. Other proteolytic systems including calpains, proteasome, cathepsins, matrix metalloproteases, thrombin, plasmin etc … will then participate to the cell dismantling process but we still do not know in what order and to which extent, even if some of them are suspected to be able to activate some caspases at least *in vitro*. In this respect, we must keep in mind that *in vitro* successful protease substrates are not necessarily *in vivo* target substrates of the protease considered.

On the other hand, quality markers would be a reflection of the different metabolic pathways contributing to the *postmortem* development of meat tenderness. Hence, a better understanding of these pathways and their interactions is a prerequisite for a successful identification of accurate biological/biochemical markers of this primary quality attribute of meat. In this respect, the advent of modern proteomic technologies has undoubtedly contributed to a better understanding of these processes (D'Alessandro & Zolla, 2013).

In this review, we will first try to update the mechanisms responsible for the *postmortem* improvement of meat tenderness with some new features according to research carried out during last years. Then we will overview the potential markers of meat tenderness identified so far and the biological structure(s)/pathway(s) to which they are related. Finally, we will conclude this report by a series of perspectives to improve our knowledge about these mechanisms and the most interesting points to be investigated in the near future for a more efficient search of the best biomarkers of meat tenderness.

2. Meat tenderization mechanisms: some new features

As commented above, meat tenderization is a complex process still not clearly understood. With refinement of the techniques, a large set of new features have been reported during last years, thus making the actual concept of meat tenderization somewhat confusing and much more complex than expected. Nevertheless, we think that there is a consensus on the two features commented above: (1) the multi-enzymatic nature of meat tenderization, and (2) the onset of apoptosis as the first step in the conversion of muscle into meat.

2.1. Major events following animal bleeding

After animal bleeding, tissues come into an ischemic anoxic state which will affect all metabolic pathways and will lead to an adaptation of most, if not all, metabolic processes (Ouali et al., 2006, 2007). In other words, the cell will develop contradictory tools for cell death or cell survival pathways (Fig. 1).

The first objective for the cell after entering into the anoxia state will be to improve its capacities to provide the energy needed for increased metabolic activities. As observed by quantitative analysis of 2D gel spots, this led to an increase in the enzymes associated to glycolytic and tricarboxylic acid (TCA) cycles. A major consequence of this intense metabolic activity will be the accumulation of diverse harmful byproducts including CO_2, HCO_3^-, NH_4 and lactic acid, which are normally transported to the liver where they can be recycled. Later in the context we will consider the energy aspects in the last part of this review as it could be a good source for finding new biomarkers of meat tenderness.

The second step after animal bleeding will be the preservation of cell functions by an increase in the concentration of several Heat Shock Proteins (HSPs) including HSPs 70, 40, 27, 20, αβ-crystallin, and probably others.

The battle between cell death and cell survival will finally turn to the advantage of the cell death process with the well-known characteristic changes associated to this status, especially cell shrinkage, and phosphatidylserine externalization, together with mitochondria alteration (Becila et al., 2010; D'Alessandro & Zolla, 2013; Ouali et al., 2007). A set of pro- and anti-apoptotic proteins will be released from mitochondria and their ratio will define the rate and extent of apoptosis development. For the same reason than for the energy