THE USE OF EXOGENOUS ENZYME IN MEAT TENDERNESS: CURRENT PATTERN - REVIEW

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Abstract

The texture of the meat reckons on many factors and is mutually dependent on the post-mortem period (natural aging - keeping the meat at refrigeration temperatures for a few days or weeks), a process that allows the meat to become tender under the action of proteolytic enzymes inside the cells. The emphasis of these enzymes involves glycolytic activity, a process that involves the metabolism of glycogen producing lactic acid and causing a decrease in muscle ph. Among the most important enzymes involved in the meat maturation process mentioned in the literature are the calpain-calpastatin system and the cathepsin-cystatin system. Meat aging by exogenous enzymes involves the use of plant-derived proteolytic enzymes. Over time, several proteolytic enzymes of plant origin have been used which have the potential to improve meat texture. Among the most commonly used are papaya (from papaya), bromelain (from pineapple), zingibin (from ginger), ficin (from figs/fruit), and actidine (from kiwifruit). The use of exogenous enzymes is of real importance for obtaining high-quality products that meet consumer standards for tenderness in meat products due to their technological properties.

Key words: *plant enzymes, tenderness, meat, papain, bromelain*

INTRODUCTION

Meat quality covers a concept with multiple valences in which its value is determined by several criteria: organoleptic (color, juiciness, texture), ethical and social (organic versus conventional, animal welfare), symbolic and cultural, nutritional, functional and criteria corresponding to safety, availability and price [1].

Meat tenderness is a key factor on which co-consumer satisfaction and acceptability, recurrent purchase of a product, and also the willingness to pay a higher price depend [2,3,4]. The texture is influenced by biological and extrinsic factors. In particular, the post-mortem maturation process is characterized by proteolysis of myofibrillar proteins, the amount and solubility of connective tissue, and the amount of intramuscular fat [5,6]. Warner et al. [3] mentions a close link between meat texture and molecular factors in relation to glycolysis, Ca^{2+} release, protease activation, apoptosis, and heat shock. Most of the methods used to improve meat tenderness also have an impact on myofibrillar proteins and collagen.

From a buying perspective, the key factors that lead to an ultimate acquisition in terms of meat are color, appearance, and smell [7].

The purchaser will analyze the meat by its appearance, therefore the fibrous looking on the surface of the muscle indicates the integrity of myofibrillar proteins, thus a slice of tougher meat [8]. Red meat, in particular, presents a marbled appearance, due to the presence of intramuscular fat, and can be distinguished as tender meat. Furthermore, differences in color

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characteristics of meat with a rougher texture were identified due to increased myoglobin content and the presence of "creases" on the meat surface using confocal microscopy [7,9].

The use of exogenous proteolytic enzymes in meat is a commonly used method to reduce the toughness of meat, the final appearance and texture differ depending on the type of enzyme, the amount used, and the method of application [7].

Proteases lead to the breakdown of connective tissue proteins by hydrolysis of peptide bonds [10]. Enzymatic operations include the following: infusion, marinization, or injection. These enzymes act on myofibrillar proteins and connective tissue to different lengths in order to produce a more fragmented and disintegrated structure. Proteases exhibit wide diversity in terms of protein action, structure, and properties [11]. They can be extracted from plants and / or microorganisms and are widely used.

Papain is a cystine protease obtained from the latex of the papaya plant [12]. It is used due to its ability to unravel both myofibrillar proteins and connective tissues. Papain has an excellent ability to operate over a wide temperature and pH range (3 - 9), which justifies its applicability in various industries [13].

Bromelain, a substance extracted from the stem of the pineapple plant, is used in the meat, baking, and brewing industries. In the pharmaceutical industry, it has an anti-inflammatory role and can be applied preventively for diseases such as bronchitis, sinusitis, and thrombophlebitis. In meat technology used as a powder increases nutritional value, improves texture, and decreases shear force in beef. The enzyme has optimum values of action at temperatures between 10 - 20°C in an acidic environment, and 30 - 40°C in an alkaline environment, while at neutral pH values the temperature should be between 40 - 60°C [14,15].

Another source of exogenous protease is ginger (*Zingiber Officinale*) which breaks down major muscle proteins and has gained substantial interest due to its collagenolytic activity [2,16].

The study of the applicability of by-products resulting from peeling mango fruit has led to the identification of beneficial compounds (antioxidants, polyphenols, carotenoids), including enzymes, and serine protease. Significant amounts of serine protease activity ranging from 4.573 – 11.173 U/g were identified following drying application [2,17].

Ficine is isolated from the latex of the fig tree *Ficus carica* and is used in meat tenderizing because it has the ability to hydrolyze peptide bonds at the level of aromatic residues [18].

Extracted from the kiwi fruit (*Actinidai deliciosa*) actidine breaks down animal protein and improves digestion. Used in amounts of 0.5 mg/100g in muscle, it has been reported to decrease shear force in pork and rabbit meat. Whereas in beef it showed a high ability to hydrolyze connective and myofibrillar tissue [19,20].

The present paper aims to conduct retrospective research on the influence of proteases derived from plants on the textural quality of meat, their methods of application, and their action. In compliance with the purpose of the study, distinct articles will be reviewed to highlight a clear picture of the applicability and efficacy of the use of exogenous enzymes.

MATERIAL AND METHOD

Figure 1 presents the PRISMA schematic flowchart of the elected articles. In order to achieve the objectives of this study, several bibliographic sources in the literature were reviewed and consulted. The electronic research was carried out in the scientific "libraries": Multidisciplinary Digital Publishing Institute (25), Science Direct (56), Web of Science (18), and Research Gate (28), and have been selected based on keywords ("meat tenderness", "exogenous enzymes", "plant enzymes", "texture", "proteases", "meat enzyme", "ficin", "papain", "bromelain") summing up 127 articles. From the total, papers found to be duplicated or triplicated (33) were excluded. The next stage of sorting was to read the titles and abstracts from which 44 papers were eliminated. Of the 44 articles considered of interest, 25 were included in the present study. Moreover, some papers presented were chosen for a better understanding of some of the enzymatic, technological, and physicochemical processes underlying the meat industry. The research methodology was based on analysis, observation, and interpretation of data from studies published in recent years on the influence of exogenous enzymes on meat texture.

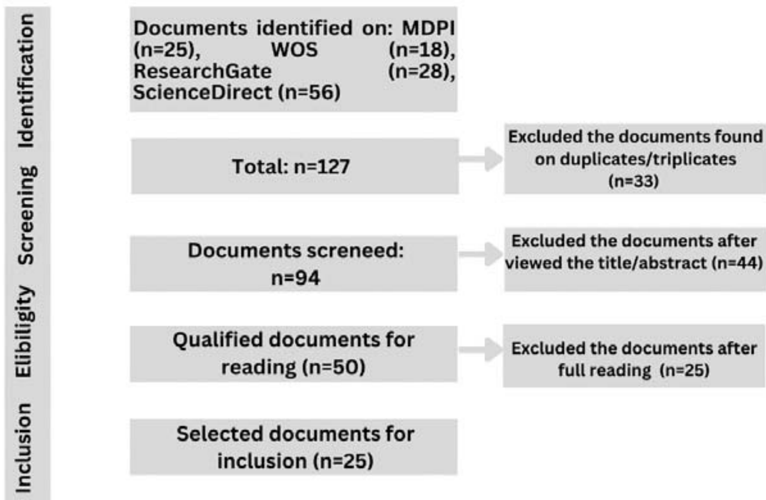

Fig. 1 Prisma schema chart for the research, election, and inclusion of the articles

RESULTS AND DISCUSSIONS

Meat quality is a term used to describe sensory properties and attributes. It includes attributes that provide for carcass quality and composition, nutritional value, and food safety. All of these factors combined are then evaluated by the final arbiter, the consumer [21].

In addition, meat quality is given by sensory and technological characteristics for example hardness, color, water-holding capacity, and texture. The attribute of "tender" meat is perceived as the most important factor influencing taste perception and buyer satisfaction. In the following, the most recent research on the influence of vegetable proteases on meat texture will be presented [7,10].

Doneva et al. [22] conducted a study aimed at evaluating the influence of immersed plant enzymes (kiwi fruit and ginger root extract) on rabbit meat (*Biceps femoris*). Differences were observed for the following physicochemical parameters: pH, water holding capacity, cooking losses, and free amino acid quantity.

Differences in the values of these characteristics were established both between control and test variants and between treatment durations. In the case of raw rabbit meat samples treated with papain and homogenized with ginger and kiwifruit, the percentage of water-holding capacity increases with increasing treatment duration. Statistically significant differences in free amino acid concentration were observed between control and test samples, with the highest values for papain-fattened samples. The electrophoretic profile of the control meat samples and the braised meat samples shows a reduction in the intensity of actin and myosin bands in all variants treated with papain and plant extracts. Test results show that ginger and kiwifruit extracts can be successfully used for the enzymatic tenderization of rabbit meat.

Zhang et al. [20] studied the extent to which actidine influences the texture of rabbit and pig *Longissimus dorsi* muscle. Thus, muscles were cut into pieces (5 x 2 x 1, length x width x thickness) and injected with vegetable protease at a ratio of 1:10 (mL sample volume to muscle weight). Subsequently, the experimental samples were packed and stored at room temperature for three hours. The samples were subjected to heat treatment in a water bath at 70°C, for 30 minutes and cooled. The results obtained revealed that the use of purified actidine in a dose of 0.5 mg/100g muscle resulted in low shear force values and improved texture. In a further use of actidine, Zhu et al. [23] followed its application to beef brisket followed by thermal inactivation. Injection of 5% of 3 mg/mL commercial actidine extract solution, followed by tumbling and sous vide cooking for 30 minutes at a temperature of 70°C, resulted in high acceptability, high values for flavor, juiciness, and texture, with no cooking losses, pH drops or color change.

In another study conducted in 2023 by Marino et al. [4], the effect of papain injection and/or ultrasound treatment on semitendinosus muscle texture by using a proteomic way was investigated. Thus, beef muscles were subjected to maturation at 3°C (control sample), papain infection (PI), ultrasound (US), papain injection followed by ultrasound treatment (PIUS), and ultrasound followed by papain injection (USPI). Several physicochemical indicators were analyzed at 2, 24, 48, and 96 hours after storage. The highest values were recorded in samples that were treated with papain, papain injection followed by ultrasound treatment and ultrasound combined with plant extract for soluble collagen content and myofibrillar fragmentation index. The lowest Warner Bratzler shear force (WBSF) and texture values were recorded at 48 h after storage in PI experimental samples, while at 96 h after storage meat samples subjected to USPI treatment achieved comparable results to those injected with papain. At the same time, the lowest values of cohesiveness, gumminess, and chewiness were found in the samples injected with vegetable protease during the whole period of storage. Significant muscle protein degradation and hydrolysis were observed in the papain-containing treatments. Papain injection induced intense proteolysis leading to faster muscle fracture in beef muscle. The USPI treatment, after 96 hours of storage, had the same results in terms of texture improvement as the enzyme treatment, except that it had a lower rate of hydrolysis, which is crucial in preserving the textural structure of the meat. Thus, the plant extract treatment was effective in hydrolyzing connective tissue and improving the solubility of *semitendinosus* muscle. Papain injection promotes intense proteolysis leading to an early tenderisation process and generating a large number of peptides. This may lead to reduced microstructure stability and integrity, correlating with the low cohesiveness results observed in PI samples.

Related to the article reported previously, Barekat and Soltanizadeh [24] carried out a study to improve tenderness by applying at the same time high-intensity ultrasonic radiation and papain treatment on beef muscle (*Longissimus lumborum* muscles). Equal pieces of muscle were cut (3 cm^3) packed into plastic bags and frozen at -18°C until the analysis was performed.

Two types of treatment were done: (a) ultrasonic radiation and (b) ultrasonic radiation and papain with the concentration of vegetable protease in the ratio of 0.1g/100 ml. The samples were sonicated using an ultrasonic sonde (20kHz) and exposed to different powers: 100 and 300W, for a period of time of 10, 20, and 30 minutes, respectively. Both the use of ultrasonic radiation alone and ultrasonic radiation and papain resulted in decreased shear force, filter residue, and improved texture. Significant proteolytic activity was observed in the samples where the combined treatment was used at a power of 100 W for 20 minutes.

The effectiveness of papain has also been investigated on the texture of yak (*Bos grunniens*) meat by combining papain and high-pressure processing treatments [25]. Samples injected with papain (80 U/mL) and applied a treatment (55°C, 120 min) revealed a 46.85% decrease in shear forces and a 9.93% percent increase in water holding capacity, with negatively correlated changes for color. In comparison, samples that were injected with plant extract (80U/mL), incubated (55°C, for 30 minutes), and subjected to high-pressure processing (50Mpa/15 minutes) showed positive effects on color, better texture, and water-holding capacity.

Maximizing the impact of pineapple by-products rich in bromelain by using hydrostatic pressure (225 MPa, 8.5 minutes) on the texture of silverside beef cuts was studied [26]. Samples were cut 2 cm thick, weighing 270 g, and immersed in marinades to which pineapple by-products correspond to a bromelain concentration of 0 - 20 mg tyrosine. 100 g-1 meat, for 0 - 24 h. The samples were then subjected to heat treatment (80°C, 15 minutes) and stored for 24 hours at 4°C. The results showed that marinating (12 - 24 hours) and bromelain concentration (10 - 20 mg tyrosine*$100g^{-1}$ meat) reduced the pH and hardness of beef, increased the marinating yield, and, the brightness values in the color analysis were higher. Although keeping samples at refrigeration temperatures (4°C) was not optimal for activating plant protease, the percentage of meat toughness decreased by up to 41%.

The influence of bromelain powder, extracted from the core of the pineapple plant, was analyzed by Nadzirah et al. [27], in 2016 on the texture of beef round cuts. The samples were divided into two groups: a control group and a group to which bromelain treatment was applied. The 2 cm^3 meat cubes were immersed in a 0.17% bromelain solution at 60°C for 10 minutes. The results of the research revealed a decrease in meat toughness and a significant increase in the content of essential and non-essential amino acids in beef. On the other hand, it led to the reduction of water holding capacity value and an increase in pH.

The meat tenderizing process is mainly based on the use of enzymes of plant and microbial origin. Abdel-Naeem et al. [28] have focused on investigating the impact of extracts of plant origin (ginger and papain) on structural, qualitative, and electrophoretic pattern changes in camel meat (*Camelus dromedarius L.*). The camel meat analyzed was cut evenly (3 x 3 x 3) with fat and connective tissue removed in advance. Four experimental samples were prepared: (I) control sample: pieces of meat mixed with 14 mL of distilled water; (II): 7% (w/v) fresh ginger extract, pieces of meat, and 8 mL of distilled water; (III): 0.7% papain powder (g/g): meat pieces, 0.7g papain powder, and 14.3 mL distilled water; (IV): 0.5% (w/v) fresh ginger extract and papain powder (0.5%) sample and 9.5mL distilled water. All samples were packed in polyethylene bags and stored at 4°C and marinated for 48 hours. According to quality analysis, the best results were observed in meat samples treated with fresh ginger extract mix and papain powder. The writer justifies the increase in protein solubility by increasing the permeability of connective tissue and myofibrillar proteins, resulting in easier disintegration. At the same time, the plant enzymes act on the intermolecular cross-links of collagen which causes structural changes. All experimental variants showed higher values in comparison to the control sample for cooking losses and lower pH values. The significant increase is due to the proteolytic effect of enzymes of plant origin that favor protein denaturation. Shear force values showed decreases for variants treated with fresh ginger extract and papain powder. The property of exogenous enzymes to solubilize collagen results in meat with a more tender texture.

Naqvi et. al. [29] studied the influence of injected ginger powder combined with sous vide cooking on beef (*M. biceps femoris*) sourced from old animals. Before preparation, samples were thawed at 2 - 4°C for 48 hours. After slow thawing, fat and connective tissue were removed and cut into halves parallel to the muscle fibers. The control sample was vacuum-packed and stored at 4°C. Equidistant injection of ginger powder into the muscle at a depth of approximately 3 - 4 cm involved obtaining an aqueous solution of concentration 2g/L, which was then vacuum packed and stored at 4°C, for 24 hours. Samples were cut (2.5 x 3 thick) and sous vide cooked for 8 and 12 hours at 65°C. The trial results revealed that vegetable proteases from ginger combined with sous vide cooking can improve the tough texture derived from old animals. The values obtained from the sensory and physical analysis revealed that too long cooking time (12 hours) of the injected samples resulted in meat with a too-soft texture. The best value for the parameter "crispness" in the sensory analysis was obtained for the sample injected and heat-treated for 8 hours. The value also correlated positively with the results obtained for shear force for both injected and sous vide treated samples. This indicates that the vegetable protease used has the property of improving the texture; at the same time, the low values obtained for the control samples (injected with water) reveal that it is not due to the tearing of the meat by means of needles that a lump of more tender meat was obtained.

CONCLUSIONS

Plant proteases can be used as possible "tools" in the value-adding of meat products with low economic value, improving sensory parameters (tenderness, juiciness, chewiness) and nutritional value. The use of exogenous enzymes of plant origin has been shown to be effective in improving meat texture. In spite of that, certain parameters need to be well established such as optimal temperature and pH of activity, quantity, and method of introduction, as well as the type of treatment used.

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