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Highlights

- Plant-derived proteases used to enhance the texture of meat products
- Plant cysteine proteases used as exogenous tenderizing agents of meat
- Current studies and main conditions of plant proteases as meat tenderizing agents
- Meat tenderized using plant and fruit-derived proteases can target elderly people

Abstract

The use of cysteine plant/fruit-derived proteases in meat tenderization enhances the overall texture and palatability of meat, especially beef. Tenderization with natural plant proteases is relevant for consumers with regard to nutritional, healthy and sensory properties and for meat industry stakeholders as it affects their profitability and allows diversifying the range of their products by adding value to low-value meat cuts. Current researches on the use of plant proteases as a green and sustainable approach to enhance meat texture were presented. The main cysteine plant proteases investigated/applied as tenderizing agents of meat were described; followed by the methods of their application alone or in combination with processing treatments; next, their emerging use to produce protein hydrolysates from meat or for ensuring better meat digestibility was presented and finally their potential for texture-modified meat products targeting elderly consumers was summarised.

Keywords: Plant enzymes; Meat and beef texture; Muscle proteins; Elderly consumers; Proteolysis; Peptides

Introduction

Tenderness is the main quality attribute, regardless of the species and muscles, used by consumers to judge the overall acceptability of meat and meat products as well as for making re-purchasing decisions. When meat is of guaranteed tenderness and its qualities correspond to the expected preferences, the consumers are willing to pay more [1]. However, meat tenderness is known as a multifactorial sensory attribute even among animals sharing the same genetic and reared following identical practices, indeed a substantial biological and uncontrolled variation exist [2]. Within muscles, tenderness is a result of sophisticated and interconnected pathways [3], also associated with the amounts of fat and connective tissue, length of sarcomeres, ratio of the proteolytic systems/endogenous inhibitors, etc. This inherent tenderness variability among meat cuts is mostly understood by consumers associating it with the methods of cooking or to the price of the meat cuts themselves. However, inconsistences from the same meat cut, especially the valuable pieces, are a serious concern for the meat industry stakeholders and consumers. Therefore, several *post-mortem* processing interventions, along with enzymatic treatments using exogenous proteases, were investigated to enhance meat tenderness and reduce its variability [4, 5]. Although the bulk of commercial proteases is produced mainly from microbial and animal sources, the potential of plant sources, especially plant cysteine proteases, are increasingly used in food/meat industry [6].

The use of plant proteases to produce consistent tender meat, by making the entire carcass more valuable, can be considered as an emerging sustainable option to retain consumer confidence, especially in red meat [7-9] or to add value to low-value meat cuts, to sell consistently guaranteed tender meat products and also as an alternative to reduce food industry waste in the frame of circular economy [10, 11]. The best example to cite for this latter objective is the recovery of bromelain from peels and core of pineapple processing waste [12, 13]. Further, the interest for the use of natural plant proteases was argued by technical and societal reasons. Technically, plant proteases have unique properties and activity over a wide range of pH and temperature, as well as large stability and substrate specificity that make them suitable to tenderize meat. Religiously, they conform for example to the halal consumption requirements by avoiding products from animal origin that are prohibited or the use of growth media for microbial protease fermentation that do not feat with that requirement [14]. Ethically, mainly by the use of genetically modified organisms to produce proteolytic enzymes presents controversial among consumers [15].

Based on the above and growing interest for high and guaranteed texture quality of meat, and quest for healthier meat products, this mini-review aimed to summarise the current studies using cysteine plant/fruit-derived proteases as a strategy (a) to enhance meat tenderization, (b) to develop texture-modified meat products targeting elderly consumers, (c) to produce protein hydrolysates and ensure a certain amount of bioactive peptides with healthy properties and (d) to ensure better meat digestibility (**Figure 1**).

Main cysteine plant proteases used as meat tenderizing agents

Proteases have already taken a great role in food formulation, processing, and production, especially with the advancements of biotechnology and enzymatic biocatalysis including progress in separation and purification processes over the last two decades, allowing important advantages in several industrial processing such as meat industry [16]. Among the enzymes, plant proteases and more specifically cysteine proteases (**Figure 1**) among which papain (EC3.4.22.2), actinidin (EC3.4.22.14), bromelain (from stem EC3.4.22.32), zingibain (EC3.4.22.67) and ficin known also as ficain (EC3.4.22.3) have been widely investigated as meat tenderizing agents [4, 8]. These listed enzymes are also the major proteases recently used to enhance the tenderness of meat from different species (**Table 1**). Each of these proteases has varying degrees of activity against the structural and myofibrillar proteins as well as against the collagen component. In fact, it is for their tenderizing specificities that several plant proteases were investigated towards some muscle proteins in the objective of enhancing, positively, the *post-mortem* changes of meat (**Table 2**). Among these proteases and to the best of our knowledge only papain, bromelain and ficin were for instance generally recognized as safe (GRAS) to be used as meat tenderizing agents or to produce peptides and protein hydrolysates [17, 18]. Each of these proteases has been shown to have varying degrees of effect against meat matrices, more specifically on the myofibrillar, sarcoplasmic and/or collagenous proteins, hence modifying the texture outcome of meat (**Table 1**). These proteases can also induce excessive tenderization, making the meat albeit mushy, therefore optimizing the physical conditions and duration of their use is very important.

Papain is a protease of 212 amino acids that has a molecular mass of 23 kDa [19], which is extracted from papaya (*Carica papaya*) latex. It is one of the main plant proteases used to tenderize artificially meat due to its ability to hydrolyse larger muscle proteins, with main specificity to phenylalanine (Phe) and tyrosine (Tyr) at the P2 position , into smaller fragments and peptides [20, 21]. The performance of papain is due to its activity at temperatures ranging from 40 to 80°C (maximum activity at 60°C) using casein as substrate, but with further activity for shorter time at 80°C. For example, it retained 70% of its activity when incubated at 40°C for 1 h [19]. In addition, papain showed an increase in its activity in the pH range from 5.0 to 8.0, and better stability at pH 6.5 to 7.5.

Actinidin also spelled in the MEROPS and BRENDA databases as actinidain is the 23.5 or 32 kDa protease extracted from kiwi fruit (mostly *Actinidia deliciosa*). Its concentration and molecular mass may differ among cultivars. The maximal activity of actinidin was observed at pH 5–7 and a temperature of 45°C. The low inactivation temperature of actinidin (<65°C) allow a better control of the meat tenderization process, hence avoiding high temperatures for its denaturation [22]. However, although the denaturation temperature is low, optimizing the enzyme activity by heat denaturation should be done [23].

Ficin or ficain is the protease found in the latex of *Ficus carica* fig tree and has broad substrate specificity and pH optima [8]. It is widely used in the food industry, from the artificial tenderization of meat to the preparation of protein hydrolysates [24].

Bromelain comprises a group of endopeptidases found in different parts of *Bromeliacea* family including the stems, leaves, roots as well as the fruits. The proteases from fruit (aspartic endopeptidases) and stems (cysteine endopeptidase) of pineapple (A*nanas comosus*) are the well-known and investigated bromelains [8]. From the stem extracts, which is the mainly used source of bromelain by the food industry, further minor cysteine proteases can exist, such as ananain and comosain [8]. As for papain, bromelain is also a 212 amino acid sequence including 7 cysteines, of which one is involved in the catalysis. The purified enzyme is known to be stable at storage (-20°C) for several months and has optimum activity at temperature 50–60°C and pH ranging from 6.0–8.5, respectively.

Zingibain is the protease present in the rhizomes of *Zingiber officinale* and has estimated molecular mass of 33.8 kDa [25]. The protease has maximal proteolytic activity at 60°C and pH 7.0 [26]. It is stable at temperatures ranging from 40–65°C during 2 h. In contrast to the other proteases, zingibain has more specificity on collagen breakdown [7].

From the above, we can see that these thiol proteases have broad-spectrum specificities and activity at different pH and temperatures therefore causing an indiscriminate breakdown of both myofibrillar proteins and connective tissue (collagen) proteins (**Table 1** and **Table 2**). According to Bekhit *et al.* [7], the successful use of any of these proteases lies in understanding the characteristics and strength of the protease to be used and the environment and conditions where it will be applied. Generally speaking, the underlying mechanisms of their interventions to tenderize meat should not be very different from that of the endogenous proteases to the muscle that occur through the disruption of the native microstructure of the muscle by the catalysis of the hydrolytic cleavage of the peptide bond present in proteins and peptides. Among the investigated proteases, ficin is less widely applied due to its high hydrolytic activity which often renders meat too mushy. On contrary, papain has been observed to over-tenderise the meat and produces more off-flavours, even some studies allowed to optimise the conditions of use to minimise this undesirable result (**Table 1**). At the molecular level, papain preferably cleaves peptide bonds involving basic amino acids and it also has an esterase activity. Thus, papain degrades myosin and actin at equivalent rates, whereas bromelain degrades myosin preferentially. On the contrary, bromelain act more on connective tissue (collagen) especially on the heat-denatured form of collagen (gelatine) generated during cooking (**Table 2**). Overall, bromelain demonstrated lower activity towards myofibrillar proteins compared to papain and ficin (**Table 2**). It further allows a juicier meat after treatment, with a higher water-holding capacity and less off-flavours. Zingibain and actinidin, both not yet approved as GRAS, were less investigated compared to papain and bromelain but found to target moderately myofibrillar proteins and zingibain has more activity against collagen (**Table 2**). Actinidin seemed of great interest due to its mild effect and promising use for several objectives, especially for its low inactivation temperature and the activation role it plays on calpain 2 [27], thus leading to better tenderization results. Overall, depending upon the treatment, the degree of degradation desired, the actual target as well as the final outcome of the tenderized meat, each of these five proteases has its purpose and place within the meat industry. However, these plant proteases act unfortunately more actively on other meat proteins than on collagen. Therefore, attempts to tenderize collagen-rich connective tissue inevitably led to too extensive hydrolysis of non-collagen proteins, resulting in mushy meat. Future studies are needed to better control the activity and method of incorporation of these proteases to avoid two main disadvantages being over-tenderization and generation of bitterness in treated meat.

On another hand, these plant preparations are often crude extracts with a variety of proteases with low specificity and digest as presented above all meat proteins both from the myofibrils and connective tissue. Considering their potential, nowadays we assist to several studies aiming to develop simple methods for their recovery by enhancing both the purity and activity before use as meat tenderizing agents [24, 25]. Three phase partitioning is one of the emerging tools proposed for fast recovery of plant proteases [28] and efficiently applied for meat tenderization [19]. Reverse micellar extraction is another downstream processing method used to recover bromelain and its application to reduce meat toughness [29].

Plant proteases as meat tenderizing agents alone or in combination with processing treatments

Artificial tenderization of meat using plant proteases was applied under different methods or treatments such as injection, dipping, marinating in an enzymatic solution, spraying (pulverization) with a powder or a solution of the enzyme on the surface of the meat or infusion with an enzymatic solution (**Table 1** and **Figure 1**). These treatments allow the protease to come into contact with its substrate (meat matrix). Overall, the injection method needs a lower dosage of the enzyme, due to the increased contact area, to achieve the same level of tenderization of the other methods that has a restricted enzyme–substrate contact area. The protease can be used alone or combined with one or more enzymes depending on the final objective (**Table 1**). The efficiency of several proteases at different concentrations can be compared [30]. Other methods such as high hydrostatic pressure (HPP), ultrasounds, cooking or tumbling under sous vide conditions, freeze/thaw cycle, electrical stimulation or tenderstretching of the carcasses before using the enzymes can be also combined with the listed tenderizing treatments to accelerate the efficiency of the penetration of the enzyme and its contact with the substrate, therefore enhancing the tenderizing process (**Table 1**). Indeed, the uses of plant proteases in combination with this non-exhaustive list of methods have more promising effects on meat tenderness aiming homogenous diffusion and distribution of the protease(s) in the meat matrix and consequently better control.

Barekat and Soltanizadeh [20, 31] investigated the simultaneous impact of latex papain alone or in combination with ultrasounds as a safe and non-invasive technology to tenderize beef. The application of papain, either alone or combined with ultrasound, significantly decreased the toughness of meat evaluated by Warner–Bratzler shear force (WBSF) as well as other textural traits (**Table 1**). The authors reported that ultrasonic treatments (20 kHz) at

100 W for 20 min only in the presence of 0.1% papain solution gave the best tenderizing outcome. In another study where injected papain in addition to HPP applied separately or in combination to tenderize the thigh muscle of yak at 55°C for 2h, WBSF was decreased by 46.85% and water-holding capacity (WHC) increased by 9.93% [32]. The application in this study of HPP alone (250 MPa/15 min) was effective; however the impact on colour was important and damageable on the final product (**Table 1**). It seemed that the combined and sequential treatment of papain followed by HPP impacts positively the tenderization efficiency without effect on colour. In the same approach, another study investigated ficin at different concentrations or in combination with HPP or with HPP alone to improve the texture of tan mutton [33]. Interestingly, the results confirmed the synergistic impact on meat quality improvement compared to the ficin enzyme or HPP alone. The authors further revealed that the increase in tenderness using the sequential treatment with the protease and HPP was mainly related to the degradation of myofibrillar and sarcoplasmic proteins. Beyond meat, the enzyme-assisted HPP approach on Chilean abalone mollusks lead to better tenderization with HPP papaya latex impregnation pre-treatment as assessed by sensorial and instrumental analyses compared to injection or immersion [34].

Camel meat is known to be tough and the use of plant proteases was proposed as an alternative [7, 19, 35]. Maqsood *et al.* [35] tested bromelain, papain and ficin to tenderize the inside round of aged female camels. By means of injection of 50 or 100 ppm of the proteases at 4°C for 4 days, the authors revealed a dose-dependent with equivalent impact of papain and bromelain on tenderness (**Table 1**). The 100 ppm papain and bromelain induce high solubility of sarcoplasmic proteins and soluble peptides and collagen, hence explaining the impact on camel meat tenderization. However, papain caused high drip loss and low WHC. Another study used efficiently latex papain recovered by three phase partitioning on *Semitendinosus* and *Longissimus thoracis* muscles of old Sahraoui dromedary males [19]. The authors applied four tenderizing treatments: immersion, injection; pulverization or freeze/thaw cycle after pulverization. All the treatments improved the tenderness of both muscles and freeze/thaw cycle after pulverization was the best strategy, while immersion treatment reduced WHC [19]. In the frame of meat formulation, the physic-chemical and sensory properties of camel meat burger patties were improved by 7% of ginger extract, 0.01% papain or a mixture of both [36]. This strategy allowed increasing the solubility of collagen, the scores of tenderness, juiciness and overall liking and the reduction of WBSF.

Actinidin is an emerging protease that is gaining much more interest, especially due to its interesting physic-chemical properties described above. Zhang *et al.* [37] investigated the effect of injected actinidin from several kiwifruit cultivars on *Longissimus dorsi* muscles of rabbit and pork meat. Actinidin treatment improved the tenderness of both meats using 5% of the purified actinidin. An elegant study by Lees and co-workers confirmed recently and for the first time at the consumer level the potential of commercial kiwifruit extract [38]. In this study, the striploin and outside flat meat cuts of grass-fed steers were infused with actinidin before grilling and roasting after aging. Compared to the controls, actinidin improved significantly the consumer scores of tenderness, juiciness, flavour and overall liking. The MQ4 score (MSA overall eating quality) was also improved, therefore the use of actinidin kiwifruit extract to beef is an opportunity to improve the eating experiences for consumers [38]. Zhu and colleagues used hot boned bovine brisket muscles from dairy beef that were injected with 5% of actinidin extract (**Table 1**) followed by vacuum tumbling and cooking under sous vide (70 °C/30 min) [39]. These resulted in a significant improvement of tenderness, juiciness and flavour of actinidin-treated meat evaluated by sensory panellists compared to untreated samples. The enhanced texture was supported by considerable breakdown of the myofibrillar structure, mainly Z-discs. The treated samples in this work had no significant change in pH, colour and cooking loss. Another study tested the hypothesis of combining actinidin with electrical stimulation and tenderstretching to improve the tenderness of *Longissimus thoracis* et *lumborum* muscle of entire male alpacas [40]. This study evidenced that infusion with actinidin reduced WBSF relative to control but resulted in reduced consumer acceptance. Furthermore, the authors reported high values of oxymyoglobin/metmyoglobin (oxy/met) reflectance ratio for the enzyme and water infused product than for non-infused product, indicating greater colour stability and shelf life for infused product. In fact, the reduced oxidation through actinidin infusion may link to antioxidant properties within the kiwi fruit juice. However, the authors concluded that there is no clear advantage of using actinidin for tenderization of alpaca meat in addition to processing conditions. Actinidin was also applied alone to improve the texture of chicken meat [41] or combined with tumbling to enhance the quality of chicken nuggets prepared from spent hen [42]. Overall, actinidin showed mild tenderizing activity which avoids surface mushiness.

Bromelain was also investigated to improve meat texture. Chang and co-workers used pineapple and kiwifruit extracts to investigate the synergistic effect of injection and sous-vide on pork tenderization at several temperatures and times [43]. This study resulted in significant softening using both enzymes, but actinidin was overall more effective than bromelain. Moreover, the authors reported that the combination of sous-vide and actinidin could be a good way for extending the shelf life [43]. In another study, Botinestean *et al.* [21] achieved similar tenderization findings using papain and bromelain or a mix of both (**Table 1**) to improve the tenderness of *Semitendinosus* muscles of steers. The two enzymatic treatments reduced WBSF, but papain treatments (alone or combined with bromelain) reduced more effectively WBSF but increased cook loss. In another study that compared bromelain and papain to treat frozen giant squid muscle, the treatments with both proteases resulted in a significant decrease of WBSF, hardness, myofibrillar protein content, and calcium ATPase activity [44]. Bromelain and papain were further compared to tenderize thigh horse muscle [45]. Both proteases significant decreased the hardness. In the frame of meat formulation, promising results of the effect of bromelain embedded in double emulsion was proposed in a preliminary study on the physic-chemical characteristics of pork loin [46].

In comparison to the other proteases, zingibain was less investigated in the last 3 years. Among the studies we can cite here, it was used by Cruz and co-workers to treat chicken breast muscle after injection with 5% of the protease accompanied with storage at 4°C for 24 h [47]. The authors found that the injected breasts had high fragmentation and low WBSF values. In addition, neither the sarcomere length nor the cooking weight loss was affected.

One of the drawbacks of using plant proteases is the undesirable impact on the flavour of the tenderized meat that generates bitterness. There is scarcity in the studies that used sensory panellists or specific studies that targeted this aspect to compare several plant proteases. Among the few works, a recent trial by Zhao *et al.* [48] evidenced that papain treatment generated high amounts of amino acids. The treatment by bromelain and flavourzyme (a peptidase preparation from *Aspergillus oryzae*) significantly raised the levels of odours and ketones, whereas excessive proteolysis by proteinase K and papain significantly reduced the intensities of esters and aldehydes [48].

Plant proteases to produce protein hydrolysates and bioactive peptides or for ensuring better muscle foods digestibility

Beyond the use of enzymes to tenderize meat, recent studies have witnessed growing interest on the possible role of plant proteases in helping digestion of muscle proteins in the gastrointestinal tract [39, 49, 50] and for their use to produce bioactive peptides due to the valuable importance in human nutrition/health and more specifically for having several biological activities to play on the immune system, cardiovascular diseases or metabolic syndromes [8, 51, 52]. Since the breakdown of myofibrillar proteins is associated with improvement of functional properties, plant proteases can also be used in this context as well as for enhancing their digestibility.

Bioactive peptides produced from meat can be effectively studied for several roles [8, 51], however *in vivo* studies to prove their efficacy is the current challenging task. Few studies have focused on screening the bioactivities of generated peptides using plant proteases. As an emerging approach, Bechaux *et al.* [53] proposed a workflow that should be followed for the generation of bioactive hydrolysates, for instance from porcine products, by combining *in silico* and *in vitro* (experimental data) approaches. Bioinformatics (*in silico* approaches) is a useful tool to assess the peptides released from meat digests due to the great number of possible sequences that can be produced. Therefore, an *in silico* analysis of peptides could potentially lead to a more rapid discovery and investigation of bioactive peptides released during artificial meat tenderization. In addition, the predicted peptides generated by the *in silico* approach can be analysed using online tools that allow predicting bioactivity. Peptidomics is another approach used to investigate the interplay between artificial tenderization by exogenous enzymes, among them bromelain, and the digestibility of beef *Semimembranosus* muscle proteins [50]. The authors observed significant influence of proteolysis, which acted as a pre-digestion step on digestive changes and degree of hydrolysis. A large variation in the peptidome of beef protein digests was evidenced. According to the authors, the artificial tenderization of *Semimembranosus* muscle proteins with injected bromelain increased the survival rate of peptides during simulated digestion [50]. In this context, meat treatments that used actinidin evidenced positive effects on meat protein digestibility [39]. Another study reported further evidence of the potential of actinidin extracts to increase the *in vitro* simulated gastrointestinal digestion of cooked meat proteins [49]. Actinidin have been further shown to produce angiotensin I-converting enzyme (ACE) inhibitory peptides [37]. A targeted study on the ACE inhibitory potential of peptides isolated from papain hydrolysates (<3 and <10 kDa) of beef myofibrillar proteins using *in vitro* and animal models revealed that the <3 kDa fraction showed the greatest ACE inhibitory activity establishing its potential as anti-hypertensive bioactive hydrolysates [54].

Potential of plant proteases for texture-modified meat products targeting elderly consumers

Personalisation of certain foods unlocks the possibilities for enhancing individuals' quality of life and well-being. Sufficient intake of easily digestible protein is predominantly important for elderly people, which might present weakening in muscle mass, difficulties of chewing tougher foods and difficulties with swallowing the food. The procedures used to obtain textured meat products, have a high impact on food properties, such as organoleptic properties, therefore it will be important to not deteriorate appearance, taste and smell of these category of food, in order to not decrease their appeal. In this context, Botinestean *et al.* [55] stated that the current innovation in meat products development research focusses on the overall population, while formulations targeted to tackle the specific needs, such as older consumers, are still deficient. More people are living longer, while elderly consumers demand red meat that satisfies their distinct dietary requirements, therefore providing red meat products that appeal to elderly consumers is paramount [56]. Further, there is evidence that the deterioration of mastication and/or swallowing is prevalence within the aged segment of population, and this has a high adverse impact on their food eating decisions. In this context, it is essential to evaluate the potential options to develop texture-optimized meat products which can be consumed by ageing population, and would have a beneficial impact on their health status. Among the different strategies that have been proposed, the use of plant proteases is an emerging option [21, 45, 55].

Kim and Joo [45] stated that the development of tenderized horse meat through addition of fruit proteolytic enzymes could help the elderly consumers achieve protein dietary ratio in a lesser portion size, in order to avoid the risk of sarcopenia. To produce acceptable tender meat, the authors used papain and bromelain, and examined their influence on the texture traits. All the tenderizing treatments had a beneficial impact on the tenderness of horse meat. However further investigation is needed in order to assess the acceptance of texture-modified horse meat products by elderly consumers [45].

Botinestean *et al.* [21] explored the possibilities of using papain, bromelain, and a combination of both. The authors concluded that beef steaks tenderized with papain and papain/bromelain might be considered for addition in elderly consumers' diets, however from a technological and economical perspective, it is important to highlight that an improvement in tenderization through using plant proteases may be associated with a reduction in processing yield. Furthermore, Botinestean *et al.* [55] validated a model where beef tenderized steaks had low shear force toughness, therefore reduced the chewing effort required by elderly people. Moreover, the targeted consumers preferred the tenderized steaks in comparison with the non-tenderized ones. This was confirmed by sensory analysis, as the softer steaks were found to be acceptable by a panel of 117 elderly consumers. Considering that the texture analysis was performed in the same conditions and comparable values were obtained for textural parameters, this might be extrapolated to the study that used proteolytic enzymes in order to obtain softer beef steaks for elderly consumers.

Conclusion and future perspectives

Enzymatic interventions using cysteine plant proteases to improve the texture of meat products have several interests beyond the improvement of the sensory properties of meat products. We can see from this mini-review that the use of plant-based proteases revived during the last years, and will continue in new challenging topics in the frame of bioactive peptides release from muscle foods, and formulation of tender-optimised meat products suitable for targeted consumers such as elderly. The production of protein hydrolysates with strong meat flavour is another emerging objective to be used in soups, sauces and in ready meals. The protein hydrolysates can be further applied as seasoning additives, flavour enhancers or as nutritional additives to low-protein food products. The improvement of muscle foods digestibility is another topic that is not yet vastly explored and we expect more studies in the coming years. The area of sensory evaluation and its role in assessing the acceptability and appreciation by regular or targeted consumers of the tenderized meat products will play an essential role in the future. Further, alternative methods for enzyme inactivation should be further investigated in the future to avoid use heat treatments to inhibit the enzymes. This can be through the use of some natural molecules from plants as possible inhibitors of the proteases. Before meat industry can use enzymatically tenderized meat to produce high-quality ready-to-eat products, such as sausages, further targeted studies that would combine several factors in statistical models in the frame of optimisation are needed to avoid formation of bitter hydrolysis products. Also, the shelf-life of treated meat wits plant proteolytic enzymes are worthy of investigation in a targeted manner.

Conflicts of interest

The authors declare no conflicts of interest.

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● of special interest

●● of outstanding interest

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Table 1. Brief list of the main treatments and findings from recent studies that used plant cysteine proteases, alone or in combination with processing methods, to improve the texture outcome of meat/fish products.

Protease	Source	Meat/muscle	Processing conditions/treatment	Action on meat texture and quality/main findings	Refs
Papain	Papaya latex	*Longissimus lumborum* muscle from Holstein bulls	Ultrasonic treatments (20 kHz) at power of 100 and 300 W for 10, 20, and 30 min in the presence and absence of 0.1% papain solution	- Papain, either alone or combined with ultrasound decreased the WBSF and textural traits - Ultrasonic power of 100 W for 20 min in the presence of the enzyme gave the best tenderizing efficiency	[31] and validation [20]
Papain	Papaya latex	Fresh yak meat (thigh muscle)	Papain by injection and high hydrostatic pressure (HPP), applied separately or in combination to tenderize the yak muscle	- Injected papain (80 U/mL) alone and after incubation at 55 °C for 2h decreased WBSF by 46.85%, increased WHC by 9.93% and impacted significantly colour - HPP alone (250 MPa for 15 min) was effective, but with strong impact on colour - The combined and sequential treatment of papain followed by HPP impacts positively the tenderization efficiency without damage on colour. Optimal conditions: 80 U/mL papain/55 °C/30 min + HPP at 50 MPa/15 min	[32]
Papain	Papaya latex	Chilean abalone (*Concholepas concholepas*) mollusks	Injection, immersion and HPP impregnation by papain	- Better tenderization assessed by sensorial and instrumental analysis with HPP papaya latex impregnation pre-treatment - Enzymatic impregnation pre-treatment impacts the chromatic colour coordinates (L* decreased, and a* and b* increased in the HPP papaya latex treatment)	[34]
Papain	Latex papain recovered by three phase partitioning system	*Longissimus thoracis* (LT) and *Semitendinosus* (ST) muscles from old (5 and 6 years) Sahraoui dromedary males.	Meat pieces of 3 × 2 × 2 cm in size from each muscle were treated with 4 mg of papain and subjected to 4 treatments (a) immersion; or (b) injection; or (c) pulverization, or (d) freeze/thaw cycle after pulverization. A control without enzyme was performed.	- All the treatments, improved camel meat tenderness - For LT muscle, freeze/thaw was the best treatment followed by immersion, injection and pulverization - For ST muscle, freeze/thaw cycle allowed the greatest improvement in tenderness followed by pulverization, injection and immersion - Irrespective of muscle, immersion treatment with papain reduced WHC	[19]
Actinidin	Commercial kiwifruit extract	Striploin and outside flat of grass-fed steers from MSA graded carcasses	*Longissimus lumborum*) and *Biceps femoris* muscles were treated as follow: not infused (control) and (2) infused with a kiwifruit extract before grilling and roasting after 10 or 28 days of aging	- The use of actinidin improved significantly the consumer scores of tenderness, juiciness, flavour and, overall liking - Actidinin improved also the MQ4 score (MSA overall eating quality) for striploins and outside flat - Addition of kiwifruit extract to beef is an opportunity to improve the eating experiences for consumers	[38]

Actinidin	Commercial kiwifruit extract (Actazin™)	Hot boned bovine brisket muscles (deep and superficial pectoral) from 19-month old dairy beef	Brisket steaks were injected, after optimization, with 5% of a 3 mg/mL actinidin extract, followed by vacuum tumbling (15 min, 5 rpm, 4°C) and cooking under sous vide conditions at 70 °C for 30 min followed by immediate cooling in an ice bath	- Significant improvement of tenderness, juiciness and flavour of actinidin-treated meat evaluated by sensory panellists compared to untreated samples - No significant change in pH, colour and cook loss of actinidin-treated meat - Improvement in texture was supported by considerable breakdown of the myofibrillar structure, mainly Z-discs - First evidence of positive effects of actinidin-treated samples on meat protein digestibility	[39]
Actinidin	Chinese Xuxiang kiwifruit	*Longissimus dorsi* muscles of pork or rabbit	Both crude and purified actinidin were assayed as tenderizing agents on muscle cuts (5 × 2 × 1 cm) by injection at a ratio of 1:10 (v/w) at 5 sites using a syringe.	- Favourable actinidin tenderization effect on both pork and rabbit meat - 5% of purified protease reduced by half WBSF - Ability of the protease to produce angiotensin I-converting enzyme (ACE) inhibitory peptides	[37]
Actinidin (with MVES & tenderstretching	Commercial kiwifruit extract	*Longissimus thoracis et lumborum* muscle of entire male huacaya alpacas	Carcasses allocated to either no electrical stimulation (ES) + tenderstretching (TS); or ES + TS. Treatments were not infused (control), infused with water or infused with actinidin (0.05%)	- Infusion with actinidin reduced WBSF relative to control and water treatments but resulted in reduced consumer acceptance - no clear advantage of using Actinidin for tenderization in addition to processing conditions such as electrical stimulation and tenderstretching	[40]
Zingibain	Ginger rhizome	Chicken breast (*Pectoralis major*) muscle	Injection of chicken breasts with 5% (w/v) of the protease extract before treatment by storage at 4 °C for 24 h	- Increase of MFI - No effect on sarcomere length and cooking weight loss - Increase of tenderness (decrease of shear force)	[47]
Ficin	Commercial enzyme	*Longissimus dorsi* of tan mutton	Treatments with ficin alone at different concentrations (0 – 0.25 g/L) or in combination with HPP or in comparison with HPP alone	- Ficin combined with HPP produced synergistic impact on meat quality improvement compared to ficin or HPP alone - Increase in tenderness using ficin-assisted HPP is related to myofibrillar and sarcoplasmic proteins degradation	[33]
Papain Bromelain + Mix of both	Commercial enzymes	*Semitendinosus* muscles from Holstein-Friesian steers	Samples were treated with either papain or bromelain or mix of papain/bromelain, vacuum packaged before continuous tumbling (20 min) without vacuum, and cooking in a water bath at 68°C (20 min)	- All the enzymatic treatments decreased WBSF - Papain treatments (alone or combined with bromelain) reduced more effectively WBSF but increased cook loss - The enzymatic treatments affected similarly tenderness, colour, and cooking loss - Beef tenderization with papain and papain/bromelain offer potential for inclusion in older consumers' diets	[21]

Papain Bromelain	Commercial enzymes	Frozen giant squid (*Dosidicus gigas*) muscle	Squid mantle treated with water, bromelain, or papain solution for 40 min in a 30 °C water bath. The enzyme solution was optimised using response surface methodology	- Both papain and bromelain decreased WHC - Significant decrease of muscle WBSF, hardness, myofibrillar protein content, and calcium ATPase activity - High MFI was accompanied with the release of essential amino acids following treatment with proteases	[44]
Bromelain Actinidin	Extracts of pineapple and kiwifruit	Pork fore shanks	The synergistic effect of sous-vide and injection with plant proteases on pork tenderization was assessed at several temperatures (45, 60, 70, and 100 °C) for 0.5, 4 or 8 h	- The proteases lead to a significant softening (38–60%) - Kiwifruit extract treatment was more effective (~ 60%) than bromelain and other enzymatic treatments - Most effective treatment was with sous-vide combined with kiwi-extract enzyme at 70 °C for 8 h: low changes of total microbial count, hardness, pH and colour	[43]
Papain Bromelain Ficin	Commercial enzymes	*Adductor* muscle (inside round) of female camels, 4–5 years of age	Proteases at 50 or 100 ppm were used, by means of injection, to evaluate the tenderization efficiency on camel meat samples stored at 4 °C for 4 days	- A dose-dependent effect was observed with equivalent impact of papain and bromelain (100 ppm) - 100 ppm papain cause high drip loss and low WHC - At 100 ppm papain and bromelain, high solubility of sarcoplasmic proteins and soluble peptides and collagen - Significant increase of camel meat tenderness at 100 ppm of papain and bromelain compared to ficin	[35]
Bromelain (+ other animal proteases)	Commercial enzyme	*Semimembranosus* muscles from 20 month old Luxi crossbred yellow cattle	Meat pieces were prepared for treatment using 0.5% of enzyme to a final injection of 10%, incubated at 25 °C for several times, then heat treated in a water bath for 0.5 h at 90°C	- Significant influence of proteolysis, which acted as a pre-digestion step on digestive changes and degree of hydrolysis - Peptidomics of beef protein digests revealed large variation - Artificial tenderization with bromelain (for 0.5 h) increased the survival rate of peptides during simulated digestion	[50]
Papain & bromelain (+ other animal proteases)	Commercial enzymes	Raw horse meat (thigh muscle)	Incubation of pieces of horse meat samples with 2.5g of each enzyme at different times (1 – 8h) in a water bath at 55°C	- Better results of horse meat texture after incubation with the proteases: significant decrease of hardness - Improvement of the quality attributes of tough horse meat for elderly consumers	[45]

Abbreviations: MFI: myofibrillar fragmentation index; **MSA**: Meat Standards Australia; **MVES**: medium voltage electrical stimulation; **WBSF**: Warner-Bratzler shear force; **WHC**: water-holding capacity.

Table 2. A brief comparison of the main investigated cysteine plant proteases as meat tenderizing agents and their properties (adapted from [8, 19, 24-26, 57] and **Table 1**).

Plant protease Enzyme Commission (E.C) (Source)	**pH activity**	**Temperature activity (°C)**	**Optimal temperature (°C)**	**Optimal pH**	**Impact on myofibrillar proteins**	**Impact on connective tissue (collagen)**	**Disadvantages**
Papain E.C 3.4.22.2 (Latex of *Carica papaya*)	4.0 – 9.0	40 – 80	60 – 75	6.0 – 7.0	+++	++	Over tenderization and mushy texture. Production of off-flavours.
Zingibain EC 3.4.22.67 Ginger rhizome (*Zingiber officinale*)	5.0 – 8.5	40 – 70	60 – 70	6.0 – 7.0	++	+++	Not approved as GRAS[1]
Bromelain EC3.4.22.32 (Stems of pineapple: *Ananas comosus*)	5.0 – 8.5	50 – 80	50 – 65	6.0 – 8.5	++	+++	Over tenderization and mushy texture
Actinidin EC 3.4.22.14 (Kiwi fruit, mostly *Actinidia deliciosa*)	5.0 – 8.0	40 – 60	45 – 65	6.5 – 8.5	++	+	Not approved as GRAS[1]. Reported by some researchers as allergen.
Ficin EC3.4.22.3 (Latex of *Ficus carica*)	4.0 – 9.0	40 – 70	45 – 60	5.5 – 7.5	++	+++	Over tenderization and mushy texture. Production of off-flavours.

Symbols: +, low; ++, medium; +++, high.
[1] GRAS: Generally Recognised as Safe by the US Food and Drug Administration.

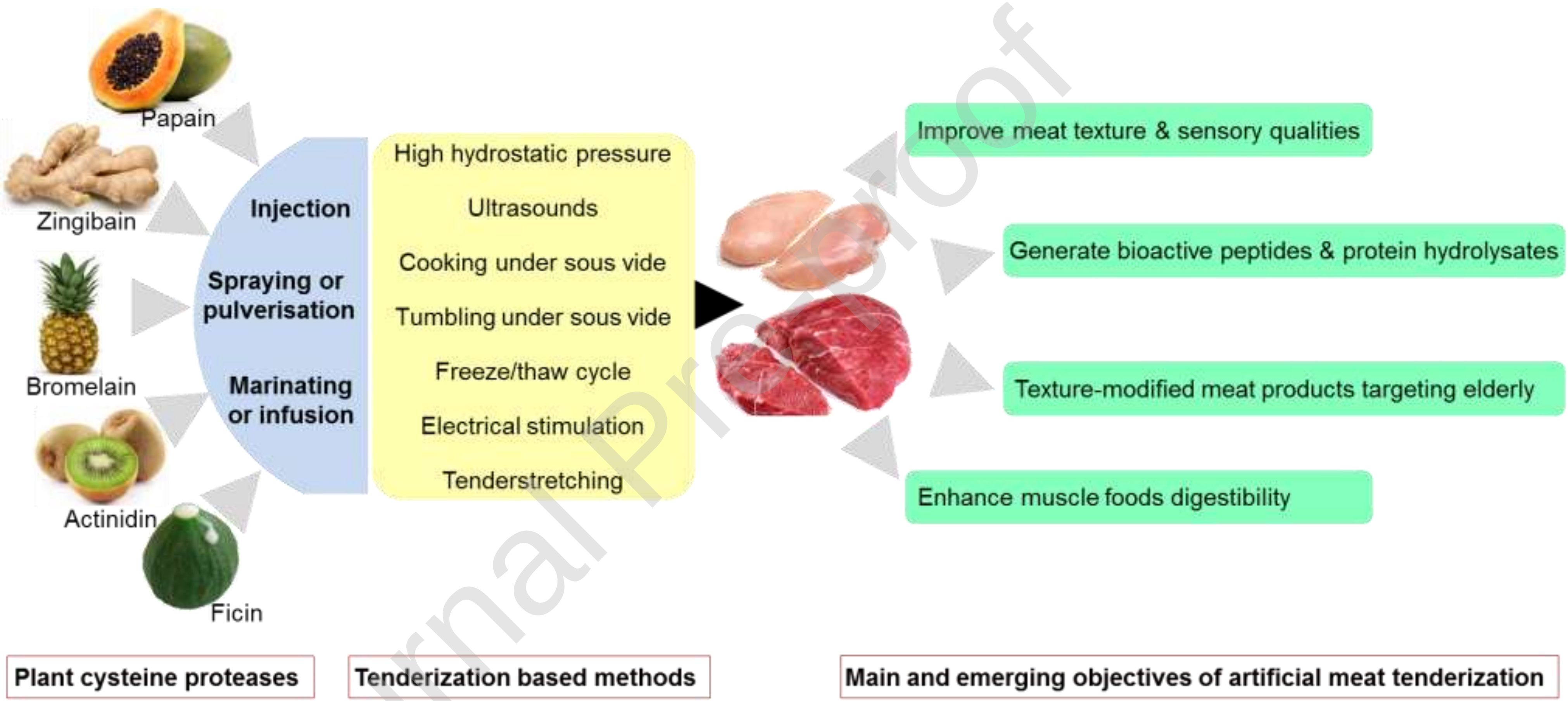

Figure 1. Main plant cysteine proteases used for artificial meat tenderization and final outcomes.