Texture characteristics of horse meat for the elderly based on the enzyme treatment

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Abstract:

Horse meat is nutritionally adequate to the elderly, but it has a comparatively hard texture in contrast to most of the food. In practice, the meat intake in the elderly is generally bated because the relatively difficult texture of the meat can diminish mastication. Thus, strategies are being developed to produce meat products remanding detracted mastication exertion and possibly exalt ingestion and nutritional stand, in the elderly. Hence, the effects of enzymes on textural characteristics of horse meat were studied, because they have well-known favorable efficacy on the meat tenderness by causing important demotion of the myo-fibrillar protein and collagen. Four treatments namely, papain, bromelin, pepsin, and pancreatin, alongside one control were invoked to the horse meat. Their effects on the texture parameters were determined. All the above enzymatic treatments significantly reduced hardness and resilience ($p<0.001$). These results, present opportunities to produce essential fatty acids fortified horse meat with soft texture and satisfied technological characteristics. The intake of the sort essential fatty acids intensified horse meat could aid the elderly to get their aimed essential fatty acid demands. Results also suggest that horse meat tenderized through enzymatic processing stand for auspicious options for the comprehension of texture-revised diets in the elderly population.

Keywords: horse meat, texture, mastication, elderly, fatty acids

Introduction

Just as the Korean committee say, by 2025 more than 20% of the Koreans will be 65 years old or above, composition of population alter that is predominantly driven by a rise in the human population aged above 80 years old (Botinestean et al., 2017). But, the particular wants of this population cohort, including ~800 million people, are repeatedly ignored by food processors, containing businesses concentrated on the advancement of novel, value-added, and easy meat products. With this next growth in the elderly population, splendid economic challenges are expected, added to the immense coercion that will be used on healthcare accommodations and related contributions. In addition, the elderly buyers do not detect with the products presently valid on the market, and the package labels neither address their wants nor accord with the awareness they have about their own health. As the elderly advance in age, their body starts to role less effectively and their prerequisite for several nutrients rises. There is, thus, a necessity for the meat field to take account of the elderly population more and to advance certain products to fulfill the broad kind of necessities in this cohort. It is important to note that one of the most crucial look towards improving the attribute of life and the wellbeing of the elderly consumers is giving them allow to joyful, safe, and nutritious food (Conroy et al., 2017a).

Nutrient-packed foods, in which prerequisite nutrients can be delivered in a comparatively little portion size, are thus, the most advisable for the elderly (Lee et al., 2007). Although meat is a precious origin of protein, vitamins, and minerals for the elderly, the comparatively rough texture of the meat can spoil mastication, thereby reducing meat ingestion in the elderly with an increasing prevail of chewing, tearing, and masticating problems, thus making them miss out on an is elderly consumers. Particularly, good quality protein foods, that are ample essential amino acids, could help commute the peril of states like sarcopenia important source of high quality nutrients (Conroy et al., 2017b). In order to avoid a nutritional imbalance, an intake of high-quality protein foods, which is of great importance in the elderly's body, is recommended. The high protein foods are important because they resume to get scientific concern for their capability to conserve svelte mass, support weight loss, and head off weight take back following weight loss or to keep a healthy weight throughout the life expectancy (Baugreeta et al., 2017).

Botinestean et al. (2016) mentioned that red meat can be a fine dietary source for elders because of its high-quality bio-available protein. Among red meat, however, horse meat is the richest in nutrients. However, raw horse meat have thicker fibers because they come from cast race or saddle horses, which are at the end of their work or slaughtered for fitness drawbacks, making it challenging to chew especially for the elderly. Besides, lack of research on the use of horsemeat can reduce the accessibility of consumers by failing to suggest how to use horse meat properly. Therefore, the diversified approach to the utilization of horsemeat would improve its accessibility of customers, and subsequently, increase the circulation of horsemeat. And the horse meat is extremely tough due to the horse's unusual diet, in spite of being delicious, rich in proteins and minerals, and low in fat (Lorenzo et al., 2013). The rampancy of chewing troubles in the elderly may be a helping factor. Therefore, there is a necessity to advance softer-textured foods for those elderly with chewing difficulties, which could play a role in counterbalancing the decay in red meat consumption. Sure enough, former studies have shown that softer textures in food products can take the lead in greater intakes by the elderly. Likewise, tenderness is a very prime property attribute by which elderly users estimate meat quality, and in the recent times when the elderly's consent for "enhanced" and processed, "meal-ready" meat products is increasing, the tenderness of such products becomes increasingly significant (Ashie et al., 2006). Besides, the Ministry of Food and Rural Affairs in Korea has quantified the traditional subjective information about chewable hardness of food for elderly in three levels. The first level of hardness of established Korea Standard (KS) is 55,000~500,000 N/m^2 and states that food is soft as elderly can chew with teeth. The second level of hardness is 22,000~50,000 N/m^2 and states that food is soft as elderly can chew with gum. The third level of hardness is under 2,000 N/m^2 and states that food is soft as elderly can chew with tongue. Thus, tenderization is essential before horse meat can be ideally made use of a food resource for the elderly (Korean Agency for Technology and Standards, 2013).

Nowadays, many ways are used to promote the tenderness of meat. Still, mechanical, chemical, biochemical, physical, and enzymatic methods have been applied by the meat processing business to attain the desired tenderness in the meat products. Among these approaches, enzyme treatments have been shown to improve tenderness with effect (Ashie et al., 2006). Especially the exogenous enzymes that are used to meat in order to improve tenderness react differently to the myo-fibrillar and connective tissue shares. These exogenous enzymes containing papain, bromelin, pepsin, and pancreatin have been extensively used as meat tenderizers. However, there is no information describing whether the enzymes have an impact on the tenderness of horse meat. This study was, therefore, undertaken to research the latent tenderizing efficacy of papain, bromelin, pepsin, and pancreatin, on the texture of horse meat, in order to comprehend their validity in the exploitation of products for the elderly or those with masticatory defects. Simultaneously, application of a microstructure approach could serve to a deeper understanding of the nature of the biochemical destructions and substitutions in muscle fiber structure and unity that are likely to be occurring at the tissue scale, during tenderization with enzymes.

Materials and Methods

Preparation of samples

Fresh race horse meat (thigh muscle) was gained from a local slaughter house in Jeju which is a part of South Korea within 24 hours after slaughter. The horse meat was cleaned with running water, and all the fat was removed, after which the horse meat was diced into rectangular slices weighting 50 g each (2 cm × 2 cm × 2 cm).

Enzyme treatment

2.5 g of each enzyme (papain, bromelin, pepsin, and pancreatin) and 1 L of sterile water (added 0.075 % of enzyme) were mixed and left standing in a water bath until the temperature reached 30℃ to make an enzyme solution. Following which, 30 g of the enzyme solution and cut-off meat sample were placed in a vacuum pack and treated for 1, 2, 3, 4, 5, 6, 7, and 8 hours in a water bath at 55℃. All these experiments were performed in three runs with whole the four treatments studied in each run. Furthermore, an untreated sample was set as a control.

Proximate composition analysis

The proximate composition such as moisture, carbohydrate, protein, fat, and ash contents in the horse meat were investigated using a Food Scan Lab 78810 (Foss Tecator Co., Ltd., Hillerod, Denmark) following the method of the Association of Official Analytical Chemists (AOAC, 2002). To establish the calories, the horse meat was homogenized in a blender (HMF

3160S, Hanil Co., Seoul, Korea), and then the homogenate was used for caloric measurements using a calorimeter (model 1261, Parr instrument, Moline, IL, USA). Calories were showed as kcal/100g of the horse meat. Cholesterol content was established according to the method described by Rhee et al. (1982).

Fatty acid profiles analysis

To determine the fatty acid composition, the horse meat was extracted using a solvent mixture of chloroform:methanol (2:1, v/v) and then the extract was methylated using the procedure as described by Seong et al. (2019). The fatty acids were divided on a capillary column (30 m × 0.32 mm × 0.25 μm film thickness) linked to a gas chromatography (GC, Model Star 3600, Varian Technologies, Palo Alto, CA, USA). The GC condition was set as follows: 250℃ for injection port and 300℃ for detector. The free fatty acids in the horse meat were identified by comparing their retention time with those obtained from the standard fatty acids. The results were expressed as relative percentages based on the total peak area. Each horse meat was done in triplicates.

Texture analysis

Texture profile analysis (TPA) was used to determine the texture of the samples instrumentally using a texture analyzer (TA-XT Express, Stable Micro Systems Ltd., London, England). The samples went under a two-cycle compression test using 25 kg load cell. Additionally, the samples were compressed using a 50 mm diameter cylindrical probe (pre-test speed 2 mm/s, trigger force 5 g, test speed 1 mm/s, return speed 1 mm/s, test distance 7.5 mm, time 5 sec). TPA recorded the following attributes: hardness - represents the maximum force required to compress the sample at the first bite and resilience - represents the ratio of work carried out between the negative and positive force input during the first compression.

Statistical analysis

Results were evaluated using the one-way analysis of variance (ANOVA) by using SPSS version 23 (SPSS Institute, Chicago, IL, USA) at a significance level of 0.05. Tukey's test which is a single-step multiple comparison procedure, was the statistical test used in conjunction with ANOVA to find means that are significantly different from each other. Tukey's test compared the means of every treatment to the means of every other treatment; that is, it applied simultaneously to the set of all pairwise comparisons and identified any difference between two means that is greater than the expected standard error.

Results and Discussion

Proximate composition

The moisture, carbohydrate, protein, fat, and ash contents in the horse meat are stated in Table 1. When compared to the obtained results in our study, Seong et al. (2016) reported slightly greater levels of moisture (71.69 g/100 g) and protein (21.28 g/100 g) and slightly lesser levels of fat (2.56 g/100 g) for the same muscle type of horse meat. These contrasting results in between studies may be referred to the disparities in the slaughter age and the breeds of animals used. According as an erstwhile studies, it was pointed that beef with higher fat content or marbling level, usually has better eating quality and was more favored by consumers. Taking into consideration the fat levels in the thigh muscle of horse meat, more studies are needed for tenderization of horse meat and the development of horse meat products particularly for the elderly.

For the cholesterol content, the consequence of our analysis revealed much lower cholesterol level as compared to that observed by Seong et al. (2016) who reported cholesterol levels as 72.36 mg/100 g. The suggested maximum cholesterol ingestion is 300 mg per day because high cholesterol intake has been linked with a promoted risk of cardiovascular diseases such as coronary heart disease and high blood pressure as well as diabetes (American Heart Association, 2008). The cholesterol content in red meat as a whole, therefore, becomes a care for the consumers. Therefore, consuming 200 g of horse loin in this study express a cholesterol intake of 126.32 mg, which agree with approximate 42 % of the commended maximum daily cholesterol intake.

As for the calorie requirements, the outcome in this study was slightly lower than that reported in the literature (160.58 kcal/100 g) (Seong et al., 2016). These contrasting results in between studies may be referred to the disparities in the slaughter age and the breeds of animals used.

Fatty acid profile

The fatty acid profile of the horse meat is showed in Table 2. A total of 20 fatty acids were found in the horse meat. Oleic acid (C18:1n9c) is the most abundant fatty acid with a mean content of 3.140 g/100 g, and it has been shown to help in reducing gut pro-inflammatory cytokine levels and has a part in fine meat flavor and savory taste (Lee et al., 2019). The savory taste and flavor are vital characteristics for meat consumers; accordingly, a rich oleic acid content can make horse meat taste better. While linoleic acid was known to stir a negative flavor in the meat, the absolute amount of linoleic acid is less than that of palmitic acid. So its effectiveness to reduce the flavor in horse meat could be expected to be insignificant (Lee et al., 2019). Palmitic acid (C16:0) was the next most plentiful fatty acid present in the horse meat with values of 2.71 g/100g, followed by linoleic acid (C18:2n6c), palmitoleic acid (C16:1), myristic acid (C14:0), stearic acid (C18:0), linolenic acid (C18:3n3), and lauric acid (C12:0). This shows that horse meat could be a good choice of food due to its high palmitic acid content, which is the first fatty acid manufactured during fatty acid synthesis and is the precursor to other long chain fatty acids (Biochemistry 2002). As a result, palmitic acid is a prime fatty acid component in the body of animals. In humans, one study reported that it makes up to 21~30 % (molar) of human fat storage, and is a main, but greatly fluctuating, lipid component of human breast milk. Palmitate pessimistically feeds back on acetyl-CoA carboxylase (ACC), which is important for converting acetyl-CoA to malonyl-CoA, which in turn is used to add to the growing acyl chain, thus preventing further palmitate generation. In protein biochemistry, some proteins are changed by the addition of a palmitoyl group in a process known as palmitoylation which is weighty for membrane localization of many proteins. According to the World Health Organization (WHO), there is compelling evidence that consumption of palmitic acid rises the risk of developing cardiovascular disease (Kingsbury et al., 1961). The present study also shows that horse meat could be a good palmitoleic acid source, which is considered to be a healthy oil component with health benefits such as antitumor activity, lowering serum cholesterol and low-density lipoprotein, and protective effects against ventricular arrhythmias.

The horse meat is also a good source of myristic acid which is generally added co-translationally to the penultimate, nitrogen-terminus, glycine in receptor-associated kinases to confer the membrane localization of the enzyme (Lee et al., 2007). The myristic acid has a thoroughly high hydrophobicity to become contained into the fatty acyl core of the phospholipid bilayer of the plasma membrane of the eukaryotic cell. In this way, myristic acid acts as a lipid anchor in the bio-membranes (Kiyota et al., 1996). Various human epidemiological studies have shown that myristic acid and lauric acid were the saturated fatty acids most strongly related to the average serum cholesterol concentrations in humans (German and Dillard, 2010). This means that they were affirmatively associated to the higher cholesterol levels as well as raising the triglycerides in plasma by some 20 %, thereby mounting up the risk of cardiovascular disease. Although some study points to myristic acid's positive effects on HDL and thus improving the ratio of HDL cholesterol content to the total cholesterol content.

Among the fatty acids analyzed out, stearic acid is known to have little efficacy on the plasma cholesterol level, while linolenic acid and cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n3) are well known to have advantageous effects on the human health (Mensink et al., 2003). An isotope labeling research in the humans reported that the fraction of dietary stearic acid that oxidatively de-saturates to oleic acid is 2.4 times higher than the fraction of palmitic acid analogously changed to palmitoleic acid. Stearic acid is less likely to be integrated into the cholesterol esters. In epidemiologic and clinical research, stearic acid was found to be related with lower LDL cholesterol in comparison to the other saturated fatty acids (Emken, 1994). Seong et al. (2019) reported higher stearic acid (4.63 g/100 g), linolenic acid (3.60 g/100 g), and cis-5,8,11,14,17-eicosapentaenoic acid (0.02 g/100g) when the results were compared to the present study. Interestingly, 0.133 g/100g of lauric acid was detected in the horse meat in the recent study. Lauric acid has been shown to increase the total serum cholesterol content more than most of the fatty acids, but most of the increase is attributable to a growth in high-density lipoprotein (HDL). Resultantly, lauric acid has been characterized as having a more disposed effect on total HDL cholesterol content than any other fatty acid, either saturated or unsaturated (Mensink et al., 2003). Arachidonic acid is also found in the horse meat, which is metabolized to both pro-inflammatory and anti-inflammatory eicosanoids during and after the inflammatory response, respectively health (Mensink et al., 2003). Arachidonic acid (ARA)is also metabolized to pro-inflammatory and anti-inflammatory eicosanoids during and after physical activity, respectively, to encourage growth. It helps to conserve the brain from oxidative stress by activating the peroxisome proliferator-activated receptor gamma as well (Hunter and Bing 2007). In adults, the interrupted metabolism of ARA may cause to various neuropsychiatric disorders such as Alzheimer's disease and bipolar disorder. As compared to the levels of each fatty acids in the horse meat in the recent study, Seong et al. (2019) informed higher arachidonic acid in their study (0.57 g/100g). These results indicate that the differences in the fatty acid content could be made for the disparities in feeding diet used or the digestion and absorption process which influences the lipids synthesis and saving in the muscle tissues among the different species of horse.

Texture

Texture is a principal quality parameter which decides the values of sensory features of red meat. It is a complex physical feature resulting from the structure and cohesion of particles. Accordingly, tenderization is essential before horse meat can be ideally utilized as a food resource for the elderly. However, there is very restricted data in the literature, especially as aging populations introduce a growing consumer market within which horse meat could do a significant and necessary part.

Furthermore, the information provided in the literature indicates that the structure of horse meat is cohesive and compact (Stanislawczyk et al., 2019). Its consistency is relatively hard. The raw materials obtained from cast race or saddle horses is normally characterized by exceptionable fibrous-nesses and hardness. One of the causes of the above weakness of this type of horse meat is the large content of connective tissue (collagen) in comparison with the other types of raw material (Moon, 2006). Thus, tenderization is necessary before horse meat can be optimally utilized as a food resource. Accordingly, tenderization of the horse meat product which in intrinsically rich in essential fatty acids, particularly, omega 3, 6, and 9, could result in an essential fatty acid-dense product that could help elderly consumers increase their essential fatty acids. Thus, from all the previous reports, the idea of using enzyme treatments was conceived in order to tenderize horse meat for the elderly consumers.

Especially, fruit-derived proteolytic enzymes have softening efficacies on the fidelity and fiber constitution of meat, which may be of relation in developing texture-modified targeted meat products for those with mastication difficulties (Chris and Gary, 2007). Five exogenous enzymes: bromelin, papain, ficin, bacillus protease, and aspartic protease, are generally recognized as safe by the United States Department of Agriculture (USDA) food safety inspection service (Botinestean et al., 2017). Of these, the cysteine proteases papain and bromelin are the most well researched into the relation to meat tenderization. Papain and bromelin function by producing critical degradation of both myo-fibrillar and collagen proteins. Several authors have also found a beneficial effectiveness of these fruit-derived proteolytic enzymes on tenderness. However, to our knowledge, no study has been conducted so far in order to examine the effects of papain and bromelin on horse meat quality for the elderly. Thus, this study assessed the capability of elderly consumers to identify horse meat of varying texture.

Table 3 presents the texture parameters of horse muscle under papain treatment. The data shows that the values of all the texture parameters of horse meat treated by papain for 8 hours after slaughter, were lower in comparison with the control. The sample treated by papain from 1 to 6 hours had chewable hardness (55,000~500,000 N/m^2) with teeth whereas the sample treated by papain for at least 7 hours had chewable hardness (22,000~50,000 N/m^2) with elderly's gum. The following results proved that papain had equivalent effectiveness on the horse meat and could effectually be used for tenderization. In the meat industry, papain is a heat-stable cysteine protease and is often used for increasing the tenderness owing to its role in hydrolysing the myo-fibrillar and collagen proteins. On account of the breakdown of myo-fibrillar proteins is connected with a betterment in the functional characteristics, papain can also be applied for this object (Barekat and Soltanizadeh, 2019). Taken together, the TPA values suggest that treatment with papain could be taken into account as a promising option for advancing softer meat products. Therefore, optimization of various tenderizing treatment options has a latent to promote the appeal of less tender cuts to older consumers and broaden the visions for those with mastication impairment.

Treatment with bromelin is also one of the most gradual methods used in meat tenderization. Along with papain, bromelin is preferable to bacterially derived enzymes mainly because of safety problems, such as pathogenicity, or other disadvantageous effectiveness (Sharma and Vaidya, 2018). This enzyme is derived from the stems of pineapples, and it helps in digesting the muscle protein when combined with the meat. It can also hydrolyze collagen and elastin, which decreases the toughness of meat (Weston and Rogers 2002). However, the amount of enzyme needs to be optimized because an excessive volume would result in meat decomposition. There is a greater extent of research on the use of bromelin on meats such as beef, pork, and chicken, but none reporting on its use on horse meat (Istrati 2008). Therefore, this study also focused on the application of bromelin in the tenderization of horse meat for the elderly consumers.

Table 3 presents the texture parameters of horse muscle under bromelin treatment. The data show that the values of all texture parameters of horse meat treated by bromelin for 8 hours after slaughter were lower in comparison to the control. The sample treated by bromelin from 1 to 6 hours had chewable hardness (55,000~500,000 N/m^2) with teeth whereas the one treated for at least 7 hours had chewable hardness (22,000~50,000 N/m^2) with elderly's gum. These results proved that pineapple is possibly a substitute source for proteolytic enzymes (Sharma and Vaidya, 2018). The pineapple is very famous in human diet due to its nice taste and high content of vitamin C, minerals (potassium, phosphorus, iron), and low calorific value. Pineapple is also a fine sources of folate, potassium, and large amounts of vitamin E. But there is little information expressing whether the proteases included in the pineapple have an influence on meat tenderization. The purpose of this study was to investigate the effect of pineapple's protease enzyme on horse meat tenderization.

Along with papain and bromelin, pepsin also do a significant role in meat tenderization and in the production of fermented foods by moulds from soybeans, rice, and other cereals (Temiz et al., 2017). It is also used in the baking industry for the modification of wheat proteins in bread production, and in the dairy industry for the clotting of milk to produce cheese. Therefore, another purpose of the present study was to find the properties of pepsin in the tenderization of horse meat for the elderly.

Table 3 presents the texture parameters of horse muscle under pepsin treatment. In this research, values of all texture parameters of horse meat treated by pepsin for 8 hours after slaughter were lower in comparison with the control. The sample treated by pepsin from 1 to 6 hours had chewable hardness (55,000~500,000 N/m^2) with teeth whereas the one treated for at least 7 hours had chewable hardness (22,000~50,000 N/m^2) with elderly's gum. These instrumental measures supply mutual complementary data on texture. While shear force is a nice measure of first bite tenderness, TPA gives a more accurate information on the textural properties of the products (Botinestean et al., 2016). For the elderly, the most critical parameters are likely hardness. The information studied in this research for TPA suggests that the enzyme treatment is a promising procedure in meat tenderization, thus recommending the elderly to promote their horse meat consume. Henceforth, pepsin can also be considered as an effective tenderizer of horse meat for the elderly consumers, which will also advantage the horse meat end-users in the market place by having more tender horse meat.

Unlike papain and bromelin, pancreatin is a mixture of several digestive enzymes manufactured by the exocrine cells of the pancreas. It is made up of amylase, lipase, and protease (Whitehead, 1988). This mixture is used to treat states in which pancreatic secretions are lacking, such as surgical pancreatectomy, pancreatitis and cystic fibrosis. Pancreatin is an effectual enzyme supplement for substituting the missing pancreatic enzymes, and aids in the digestion of foods in the cases of pancreatic insufficiency. Pancreatin also diminish the assimilation of iron from food in the duodenum during digestion (Bhattacharjee et al., 2013). They are on the World Health Organization's List of Essential Medicines, the most effectual and safe medicines needed in a health system. Therefore, this study also focused on the application of pancreatin in the tenderization of horse meat for the elderly.

Table 3 presents the texture parameters of horse muscle under pancreatin treatment. In this study, values of all the texture parameters of horse meat treated by pancreatin for 8 hours after slaughter were lower in comparison with the control. The sample treated by pancreatin from 1 to 7 hours had chewable hardness (55,000~500,000 N/m^2) with teeth whereas the ones treated for at least 8 hours had chewable hardness (22,000~50,000 N/m^2) with elderly’s gum. In another study, the enzyme treatments on beef steaked significantly reduced the TPA values (Botinestean et al., 2017). These observations are similar for the muscle fiber dispersion and meat hardness. For example, enzyme can give onto physical weakening of the fiber structure while the tenderization of meat. Thus, this coming asunder of structure may also play a part in the diminution in the TPA of the enzyme treated horse meat for the elderly consumers.

And the last, Table 3 presents hardness of horse muscle after enzyme treatment. The data shows that the values of hardness of horse meat treated by enzymes for 8 hours after slaughter were lower in comparison with the control. The following results proved that papain, bromelin, pepsin, and pancreatin had even effects on the horse meat and could effectually be used for tenderization. The tenderized parts were soft. The sample treated by pepsin and pancreatin for at least 6 hours had chewable hardness (22,000~50,000 N/m^2) with elderly's gum whereas the sample treated by papain and bromelin for at least 7 hours had chewable hardness with elderly's gum. As hardness is the major factor determining the commercial value of meat for the elderly, it is crucial that hardness diverse in the samples to estimate the varying age of commercial appeal and value of the samples. Besides, the physiological processes taking part in the buccal cavity of the mouth, such as salivation (saliva flow, rate, and composition) tongue movements, and temperate interchanges between food and oral cavity do a vital role in the awareness of texture. It is also found that mastication and capacity to swallow do a vital role in the sensation of food texture. For example, Conroy et al. (2017a) reported that the 66~70 age assessor category negatively correlated sample tender for tenderness, while the 71~75 age assessor category positively correlated the same sample for tenderness. This may be explained by a decreased cognition of texture in the latter group.

Thus, the aim of the study in identifying the proper type of enzyme for each treatment without harmful effectiveness on tenderness was successful. Resultantly, there were discords in tenderness among the various treatments. For all the texture parameters, pancreatin-treated meat was the least tender sample while papain had the lowest numerical worth and the papain-treated meat was significantly tenderer than all the treatments except bromelin. But all the treatments attest to rise the tenderness of the product in comparison to the control. Depending on the system and degree of demotion craved, each has its object and place within the meat industry. Prior to deciding which enzyme to use, one must study the system in place. Factors such as raw material, holding time and temperature, other ingredients found within the brine, handling, and cooking procedures need to be assessed in order to establish which enzyme will supply the wanted outcome.

Conclusion

From this study, it was shown for the first time that enzyme treated samples revealed a better result for texture. Extension of treatment time from 1 to 8 hours effected in a diminution in the value of all the texture parameters (hardness and resilience) in the analyzed raw horse meat. Anyhow the length of treatment time in horse meat, the used enzymes (papain, bromelin, pepsin, and pancreatin) significantly reduced the values of all texture parameters of the analyzed raw materials as parallelized to the control sample.

The consequences indicate that the enzyme treatments can act as meat tenderizer with an ability to improve the meat tenderness. The treatment of enzyme is also crucial because it diminish the cooking time, fuel expenditure, and makes the horse meat soft and tits to eat or chew, especially by the elderly consumers with teeth troubles. The product tenderized with enzymes will have the latent ability for progressing texture-optimized horse meat products, demanding a lessened mastication endeavor that could be advantageous for those with chewing difficulty, such as the elderly. Additionally, the development of tenderized horse meat could help the elderly consumers achieve their target protein provision in smaller portion size, thus curtailing the peril of sarcopenia. The use of enzyme to soften the horse meat and promote on its palatability may also alter the demeanor of many meat consumers and they may opt to start selling and consuming horse meat, which is plentiful in Korea, Jeju. Thus, these finding might help the industry and the elderly consumers to improve the quality attributes of tough horse meat. It could be in the Korean horse meat industries best attention to certain all of the populations textural favors are catered for.

Though, more inquiries are needed to see the detailed alterations of the muscle fiber proteins in the horse meat during the enzyme treatment and to adapt this technology to the food industry. And it may be requisite to define the tenderness values that would be detectable and acceptable to elderly consumers. Therefore, more study is needed in this area so that guidelines could be introduced for industrial uptake. And future work could concentrate on sensory evaluation of the horse meat prepared under the proposed enzymatic procedures to establish their consent among the elderly.

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Table 1. Proximate composition in thigh muscle of the horse meat

	Content (per 100g)
Moisture	66.7 g
Carbohydrate	0.6 g
Protein	20.8 g
Fat	11.0 g
Ash	0.9 g
Cholesterol	45.3 mg
Calorie	185.0 kcal

Table 2. Fatty acid profile in thigh muscle of the horse meat

	Content (g/100g)
Capric acid (C10:0)	0.007
Lauric acid (C12:0)	0.133
Myristic acid (C14:0)	0.560
Myristoleic acid (C14:1)	0.053
Pentadecanoic acid (C15:0)	0.032
Palmitic acid (C16:0)	2.720
Palmitoleic acid (C16:1)	0.964
Heptadecanoic acid (C17:0)	0.030
Stearic acid (C18:0)	0.312
Oleic acid (C18:1n9c)	3.140
Elaidic acid (C18:1n9t)	0.016
Linoleic acid (C18:2n6c)	1.500
Linolelaidic acid (C18:2n6t)	0.005
Linolenic acid (C18:3n3)	0.280
Arachidic acid (C20:0)	0.005
cis-11-Eicosenoic acid (C20:1)	0.044
cis-11,14-Eicosadienoic acid (C20:2)	0.032
cis-11,14,17-Eicosatrienoic acid (C20:3n3)	0.010
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.011
Arachidonic acid (C20:4n6)	0.047

Table 3. Texture of the horse meat treated by enzymes and time

Mean ± S.D.

Enzyme	Texture \ Hours	0 (control)	1	2	3	4	5	6	7	8	F-value (*p*-value)
Papain	Hardness (N/m^2)	115312.52 ± 5355.29	105816.32 ± 5159.40	94890.95 ± 3140.42	79785.88 ± 3843.94	60951.92 ± 2495.22	53607.63 ± 1756.72	50705.20 ± 993.13	32465.77 ± 634.79	25123.93 ± 289.98	340.501 *** 1) (0.000)
	Resilience	0.44 ± 0.01	0.42 ± 0.01	0.38 ± 0.01	0.36 ± 0.02	0.35 ± 0.01	0.32 ± 0.02	0.27 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	143.737 *** (0.000)
Bromelin	Hardness (N/m^2)	115312.52 ± 5355.29	95028.15 ± 424.91	68867.05 ± 509.28	66136.93 ± 1324.32	61127.50 ± 391.33	53229.52 ± 1866.44	50323.82 ± 618.26	48142.50 ± 664.36	45797.03 ± 472.27	595.029 *** (0.000)

	Resilience	0.44 ± 0.01	0.28 ± 0.01	0.26 ± 0.00	0.24 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.19 ± 0.01	0.17 ± 0.01	258.632*** (0.000)
Pepsin	Hardness (N/m^2)	115312.52 ± 5355.29	107345.93 ± 2550.99	101314.85 ± 1182.36	87020.73 ± 3846.36	64872.73 ± 2234.96	56894.31 ± 1906.19	50614.55 ± 1638.09	48477.17 ± 537.79	47357.68 ± 388.35	578.170*** (0.000)
	Resilience	0.44 ± 0.01	0.40 ± 0.02	0.35 ± 0.01	0.32 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	383.500*** (0.000)
Pancreatin	Hardness (N/m^2)	115312.52 ± 5355.29	99635.78 ± 2119.97	94592.87 ± 1370.19	67232.90 ± 1161.24	64497.07 ± 1045.79	62001.33 ± 176.97	60215.28 ± 804.91	50349.95 ± 612.34	47630.86 ± 998.07	340.501*** (0.000)
	Resilience	0.44 ± 0.01	0.36 ± 0.01	0.32 ± 0.02	0.27 ± 0.01	0.22 ± 0.01	0.19 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	143.737***

										(0.000)
F-value [2]	0.000	182.118 ***	394.303 ***	110.088 ***	93.925 ***	62.112 ***	31.774 ***	58.403 ***	639.637 ***	
(*p*-value)	(1.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	

[1] ***, p<0.001 (The *p*-value is the probability of obtaining test results at least as extreme as the results actually observed during the test, assuming that the null hypothesis is correct).

[2] Enzyme effectiveness of hardness of the horse meat (The F-value is a continuous probability distribution that arises frequently as the null distribution of a test statistic, most notably in the analysis of variance).