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Studying process variables to obtain undisturbed shaped soft meat for people with poor oral health

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ABSTRACT

This study evaluated injection (I) and vacuum impregnation (VI) as the best methods to apply papain, and other important processing conditions (batch, aging and cooking conditions), to obtain soft meat (suitable for people with poor oral health) without disturbing its original shape. Two aging times were evaluated and four cooking conditions by immersion in soup. Meat samples were injected or vacuum-impregned (0.85 kPa) with a papain solution (5% *w/v*). After cooking, they were analyzed by the compression test, and by image and sensory analyses. The results indicated that by using both methods to apply the enzyme, the obtained meat was suitable for people with poor oral health, even if VI was the better method because it minimized the factors batch, aging and cooking condition. Therefore, the best meat processing method to obtain panelists' highest softness values and the best appreciation was employing aged meat pretreated by VI and cooked at 65 °C for 10 min.

1. Introduction

According to data from World Population Prospects: the 2019 Revision, by 2050, one in six people in the world will be aged over age 65 years (16%), which goes up from one in 11 in 2019 (9%). By 2050, one in four persons living in Europe and Northern America will be aged 65 years or more. The number of persons aged 80 years or older is projected to triple from 143 million in 2019 to 426 million in 2050 (United Nations, 2019.

Two key public health objectives for the elderly are "healthy active aging" and "compression of morbidity", designed to delay physical deterioration as long as possible. Of the many approaches required to pursue this aim, achieving optimal nutritional intake is an important and fundamental element for maintaining general health. Poor oral health status is one of the most frequent causes of malnutrition because of its effect on mastication and swallowing, and can lead to severe deficiencies in energy and nutrient intakes (Gil-Montoya, de Mello, Barrios, Gonzalez-Moles, & Bravo, 2015). Some studies highlight that edentate participants have lower hard-to-chew foods intake, including fried chicken, well-done steaks and beef, than dentate elderly participants. However, consuming an appropriate amount of meat according to the body's needs and, thereby acquiring sufficient protein intake, is essential for preventing muscle loss and, ultimately, malnutrition in elderly people (Vandenberghe-Descamps et al., 2018). Protein contribution can be done by food supplements or minced meat but both are not appetizing for some consumers (Park & Lee, 2020), which decreases food volume intake and, therefore, also increases malnutrition. So obtaining soft meat, usually identified as "solid", maintaining its visual solid appearance, could be a solution.

To increase meat softness, strategies like chemical and mechanical methods are followed. Chemical methods include post-exsanguination vascular infusion and exogenous proteases, and solubilising agents likes salt (marination) and calcium. Mechanical methods consist of grinding, blade or needle tenderisation and applying high-pressure processing (HPP) pre- or postrigour with or without heat. Applying enzymes for meat tenderisation has long since been considered. Exogenous protease enzymes, such as papain, bromelain and ficin, are widely used as meat tenderizers (Eom, Lee, Chun, Kim, & Park, 2015; Fernández-Lucas, Castañeda, & Hormigo, 2017; Singh, Shrivastava, & Ojha, 2018; Takei et al., 2015; Toldrá & Reig, 2015). Papain is extracted from papaya latex (EC 3.4.22.2) and is one of the commonest plant enzymes employed for artificial meat tenderisation for its ability to break down both myofibrillar proteins and connective tissues (Singh et al., 2018). Its application has been mainly studied on beef meat (Botinestean, Hossain, Mullen, Kerry, & Hamill, 2021) but also in pork (Garg & Mendiratta, 2006; Grau, Verdú, Pérez, Barat, & Talens, 2021), poultry (DeVitre & Cunningham, 1985), camel (Abdel-Naeem & Mohamed, 2016), yak (Ma et al., 2019) and sometimes previously treated by different techniques

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such as ultrasounds and high hydrostatic pressure to increase its effectiveness (Barekat & Soltanizadeh, 2017, 2019; Ma et al., 2019; Pizarro-Oteíza et al., 2020). Some techniques are followed to apply papain. Superficially by immersion (pulverization) is used mainly in thin steaks (Barekat & Soltanizadeh, 2017, 2019; Grau et al., 2021; Hafid et al., 2020; Pizarro-Oteíza et al., 2020). There are others like immersion in vacuum tumbling, injection and high hydrostatic pressure (HPP) for processing, during which enzyme penetration in meat is required (Gagaoua et al., 2021; Hafid et al., 2020; Ma et al., 2019; Pizarro-Oteíza et al., 2020). Another technique to promote enzyme penetration in meat could be vacuum impregnation (VI). VI has been used in many studies, but mostly in vegetables (Radziejewska-Kubzdela, Biegańska-Marecik, & Kidoń, 2014). In meat, it has been used mainly for salting meat (Aykın-Dinçer, 2021; Barat et al., 2006; Grau, Albarracín, Trinidad Pérez, Antequera, & Barat, 2011), but not for applying enzymes even though the technique allows it.

Independently of the technique followed to apply enzymes, it is important to consider variables, especially when processing is not carried out in the laboratory under controlled conditions. One of the most important variables is meat heterogeneity, which is related to meat toughness. Toughness is determined mainly by the organization and amount of connective tissue in muscle, sarcomere length and the cellular expression of intrinsic proteases, and usually by the degree of aging. However, these characteristics differ among muscles in the carcass (Veiseth-Kent, Pedersen, Rønning, & Rødbotten, 2018). This last variable is a problem for consumers because cuts of different muscles usually end up on the same commercial tray and are cooked together. This renders the cooking condition another important variable because the intensity of applied heat directly affects toughness. During heating, different meat proteins denature and bring about structural changes in meat, such as destruction of cell membranes, shrinkage of meat fibers, aggregation and gel formation of myofibrilar and sarcoplasmic proteins, and shrinkage and solubilisation of connective tissue (Tornberg, 2005).

So, the increase in the elderly population, their relatively poor oral health (related to malnutrition because of its effect on mastication and swallowing), the necessity to intake protein from meat (essential for preventing muscle loss) but usually rejected because its excessive hardness, makes necessary the study of techniques to increase its softness and appetence by avoiding its minced. Besides, it is necessary to take into account variables that are usually controlled under laboratory conditions and not by consumers, such as the heterogeneity of the raw material (meat pieces from different muscles, aging degree) and cooking conditions.

The aim of the study was to evaluate both injection and VI as the best methods to apply papain, and other processing conditions, such as batch, aging and cooking conditions, to obtain meat with the highest softness values without disturbing its original shape.

2. Material and methods

2.1. Experimental design

Fig. 1 shows the schema of the experimental design. Six batches of eight trays of meat labeled as meat for stewing (600 g each tray) were purchased on different days in a local Spanish supermarket (Mercadona, Spain). According to the producer's label, the meat on each tray was obtained from a commercial bovine piece (Eyeround, Flat Iron, Chuck tender, Sirloin cap, Bottom round roast), and was cut into cubes (about 3 cm on one side) and were modified atmosphere-packaged. Commercial samples were employed to take into account the meat heterogeneity

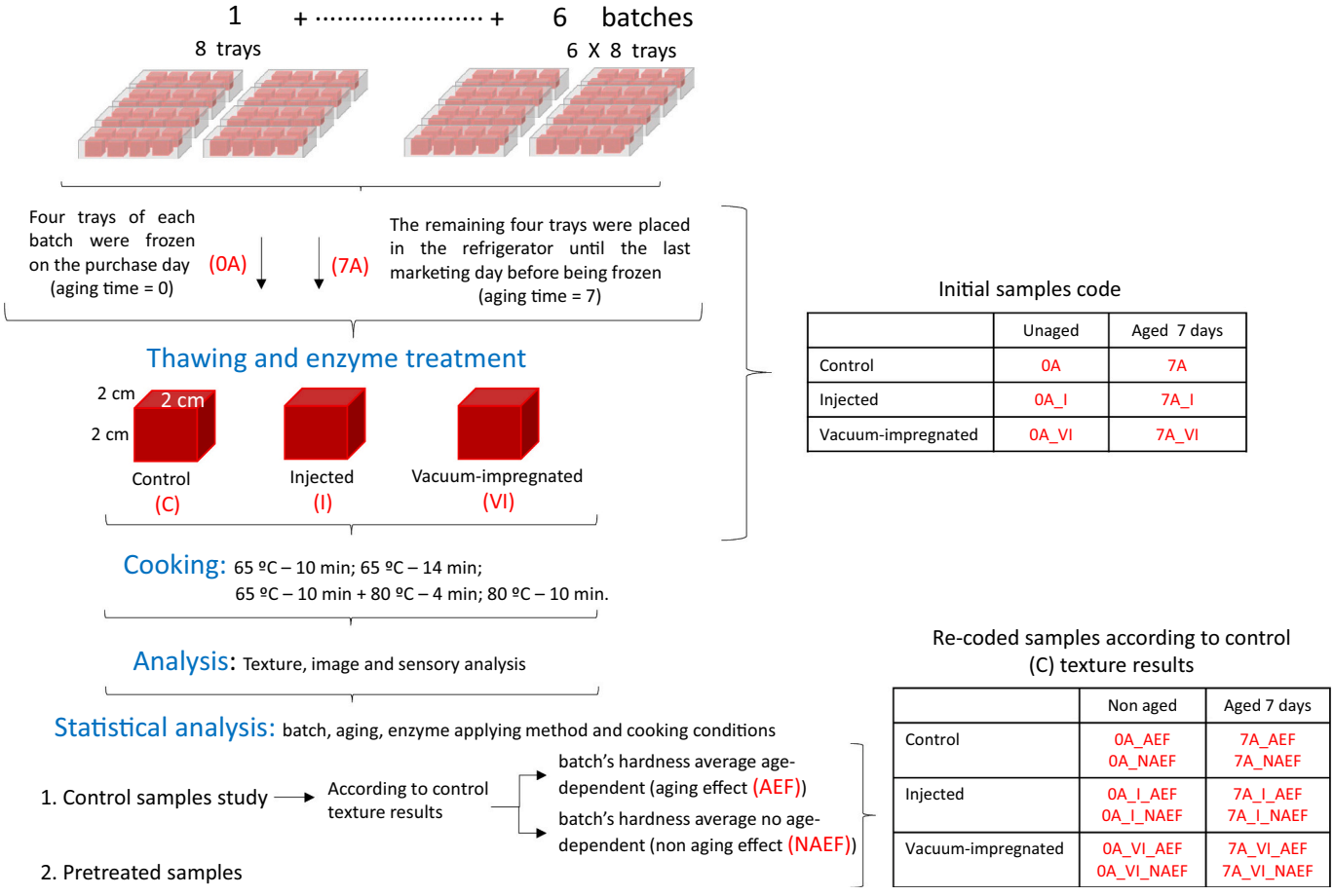

Fig. 1. Schema of the experimental design.

(meat pieces from different muscles: batch variable).

Four trays of each batch were frozen on the purchase day (aging time = 0 (0A)) and the rest were left in a refrigerator until the last marketing day before being frozen (aging time = 7 (7A)). All the trays were frozen for at least 2 days and no more than 7 days.

Once thawed, samples were cut into cubes (2 cm) to be processed as control (C), injected with the enzyme (I) and vacuum-impregnated with the enzyme (VI).

For the pretreatment, a solution (5% *w/v*) of enzyme papain (Biocon, Les Franqueses del Vallés, Spain), a proteolytic enzyme (Singh et al., 2018) with 30,000 USP activity was used. To do so, the solution was injected individually into each meat sample at a proportion of 0.1 mL every 0.5 cm. For VI, meat was placed into the papain solution (20 samples / 0.5 L) and two vacuum pulses (0.85 kPa) were applied (5 min at 0.85 kPa and 5 min at atmospheric pressure; 5 min at 0.85 kPa and 5 min at atmospheric pressure). The proportion of incorporated enzyme was evaluated for both pretreatments by weighing samples before and after pretreatment.

After the pretreatment, all samples (C, I or VI) were cooked by immersion in water at different temperatures and times which were selected according to; 65 °C – 10 min: lower temperature and time needed to coagulate the most inner proteins (time obtained on preliminary heat penetration studies in meat, in which 65 °C at the center of the samples was recorded for more than 2 min (data not shown)); 65 °C – 14 min: to explore the increase in cooking time; 80 °C + 10 min: to explore the increases in cooking temperature; 65 °C + 10 min + 80 °C + 4 min: to explore both.

So, taking into account the pretreatment (C, I, or VI), aging times (0 or 7 days), and the 5 cooking conditions, 30 different types of samples were evaluated. For each one, 20 samples were used, without taking into account those used in the sensory study.

2.2. Physical properties

2.2.1. Incorporated enzyme rate

The rate at which the enzyme was incorporated by both injection or VI was evaluated by weighing samples before and after pretreatment.

2.2.2. Texture analysis. Compression Test

A compression test was performed in a TA-XT2 texture analyser (Stable Microsystems, England). Each sample was compressed to 80% of the initial height using a resin plunger (20 mm in diameter) at a compressing speed of 2 mm/s. Stress was measured at 0–80% compression strain.

In the same way as when meat is eaten, fiber directions were not considered for the analysis and generated dispersion was assumed.

2.2.3. Image analysis

Image analyses were used to evaluate changes in samples' areas and color after cooking. For this purpose, images of samples were taken before and after cooking in a dark chamber with controlled light. The capture system was a Logitech C920 camera (Logitech.

Europe S.A., Switzerland) with CMOS sensor and resolution of 2304 × 1535. The FIJI free image software (GNU General Public License) was used to process all the images. To determine changes in areas, each meat sample was cut from the image and the number of pixels between cuts was calculated. To obtain color, the average for the red, green and blue values (RGB) of the pixels of each sample was obtained, and they were transformed into L*a*b* of CIELAB space (CIE 1976), which are normally used in food technology. Redness was calculated as a*/b* (AMSA, 2012).

2.2.4. Sensory analysis

Two preparations were evaluated: one in which only meat was evaluated and another in which meat was immersed in soup on a dish to estimate the effect of another dish component on panelists' responses. Of the 25 different treated samples, only four were evaluated by the panelists. Results of softness, from texture analysis, were the main criterion applied to select them although those from image analysis were also used.

For the isolated meat, panelists first evaluated the sample visually (overall doneness appearance and redness) and then texture by employing the spoon-by-hand method (Eom et al., 2015; Takei et al., 2015). Two samples of each treatment were evaluated by the panelists.

For dishes, panelists evaluated the overall acceptance of the images of dishes obtained by employing the previously described device. Each panelist evaluated eight images, two of each treatment, which were randomly obtained from a battery of 10 images of each dish.

All sensory analyses were performed in one session which was replicated two days. They were carried out by 33 nonexpert untrained panelists. The group of panelists was formed by 15 men and 18 women, and panelists' ages ranged from 21 to 50 years. Tests were done on a structured 9-point hedonic scale (9 = very much…. and 1 = very much….) (UNE-ISO 4121, 2003), by means of which overall acceptance (like; dislike), hardness (hard; soft) and redness (red; brown/Gy) were evaluated.

2.2.5. Statistical analyses

The effect of variables batch, aging time, enzyme applying method and cooking condition, on hardness was evaluated firstly on control samples (0A and 7A) and then on pretreated samples (0_I, 7_I, 0_VI, and 7_VI), in which the type of pretreatment was also added as a new variable. As a consequence of observed results for the texture of control samples, dependent on the batch variable, samples were re-coded according to this dependence. Two new codes were applied, one for batches aging dependent (AEF) and the other for the no aging dependent (NAEF). So, re-codes were: 0_A_AEF, 0_A_NAEF, 7_A_AEF, 7_ANAEF, 0_I_AEF, 0_I_NAEF, 7_I_AEF, 7_I_NAEF, 0_VI_AEF, 0_VI_NAEF, 7_VI_AEF and 7_VI_NAEF.

In addition, the pretreated samples were also studied individually, for injected or vacuum impregned. In both, studies were done taking into account cooking conditions of 80 °C for 10 min, and not. The effect of variables on data from image analysis obtained in control samples was evaluated as the hardness. For pretreated samples, the first statistical analysis was done in the same way but, since no batch effect was observed, they were evaluated together. In this case, studies were done individually for injected or vacuum pretreated. For the studies, multifactor analysis of variance was applied. In those cases with a significant effect ($P < .05$), the average was compared by Fisher's least significant difference (LSD).

The statistical analysis of sensory results, for the four types of samples selected according to texture and image results, was done by one-way ANOVA ($P < .05$). The session day was not taken into account.

The Statgraphics Centurion XVIII, 18.1.14. (The Plains, Virginia 20,198, USA) software was used for all studies.

3. Results and discussion

3.1. Physical properties of the control samples

For the C samples, all the three variables, batch, aging time and cooking condition had effect on hardness ($P < .001$). Broadly, hardness increased with rising heat treatment intensity, although this depended on the analyzed batch ($P < .001$) and aging time ($P < .001$) (Fig. 2A). Two main groups were observed: one in which four of the initial 6 batches had the batch's hardness average age-dependent (aging effect (AEF) = filled dots in Fig. 2A) and the other with the same statistical value (nonaging effect (NAEF) = empty dots in Fig. 2A). Taking into account this factor, for AEF batches, unaged meat had the highest hardness values in each heat treatment, and were higher than the aged meat, which had the same values as NAEF batches. Meat tenderness by aging is a complex biological process during which meat proteins

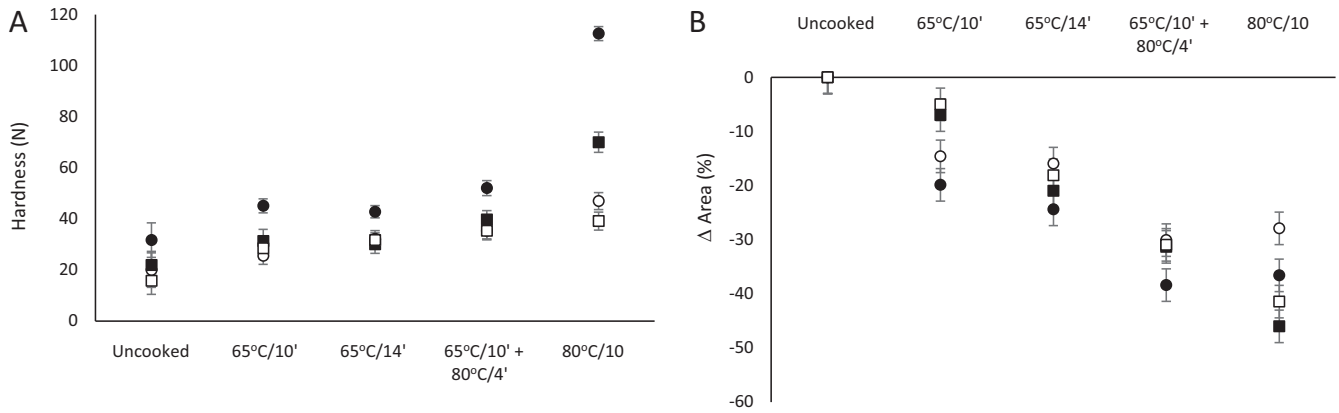

Fig. 2. Means ± standard error of hardness (A) and area changes (B) values for the control samples at each heat treatment. Filled symbol: batches of samples with different values depending on aging (AEF). Empty symbol: batches of samples whose values were equal independently of aging (NAEF). Dots: aging time = 0 (0A). Squares = 7 aging days (7A).

undergo intense degradation during postmortem aging due to the action of calpains and cathepsins, which results in increased meat tenderness (Toldrá, 2012; Toldrá & Reig, 2015). But tenderness is influenced by certain factors like the organization and amount of connective tissue, the intramuscular fat level, sarcomere shortening during rigor development, and proteolysis of myofibrillar proteins during postmortem meat storage (Della Malva et al., 2019). Therefore, the aging time effect on the tenderness of different beef muscles is not the same for them all (Della Malva et al., 2019; Nair, Canto, Rentfrow, & Suman, 2019; Veiseth-Kent et al., 2018). These authors worked with different muscles, observed how some of them, such as biceps femoris (BF), longissimus lumborum (LL) and semitendinosus (ST), displayed an aging effect on their texture, while others, such as psoas major (PM) and infraspinatus (IS), did not. Nair et al., 2019 reported a Warner-Bratzler shear force (WBSF) reduction of 38% for LL muscle while 9% for PM. These could be explaining the differences between batches of the study. In fact, for uncooked samples of batches AEF, the hardness reduction between samples with 0 and 7 aging days was 40.6% while only 5% for batches NAEF (Fig. 2A).

The effect of heat treatment intensity on hardness, dependent on the analyzed batch, was more intense for the unaged meat of AEF batches (0A_AEF). Heating at 65 °C led to increased hardness, which significantly rose when the applied temperature was 80 °C. The increased hardness was less pronounced for the remaining samples, and rose with increasing time and temperature. Changes in hardness are linked with changes in sample area (Fig. 2B), water loss and mass changes (Bruce & Aalhus, 2017; Palka & Daun, 1999). Changes in area are normally due to shrinkage or because of the loss of structure when this crumbles or dissolves during cooking, as we show later. Meat shrinkage during cooking can be described as a two-dimensional process according to temperature. Transverse shrinkage, or shrinkage perpendicular to the muscle fiber direction, takes place at cooking temperatures below 62 °C and longitudinal shrinkage, or shrinkage parallel to the direction of muscle fibers, leads to either sarcomere length or fiber length changes, which start between 55 °C and 64 °C and are completed when reaching 90 °C (; Bruce & Aalhus, 2017; Vaskova & Buckova, 2015). So, the C samples cooked at 80 °C displayed both transverse and longitudinal shrinkages, as well as more hardness (Fig. 2A) and changes in their areas regardless of time and temperature (65 °C 10′ + 80 °C 4′ = 80 °C 10′) (dots symbols in Fig. 2B). On the other hand, aged samples cooked at 80 °C for 10 min had a greater area decrease (square symbol in Fig. 2B). This could be because while myofibrillar elements become more tender with aging meat, the connective tissue is unaltered (Purslow, 2018) and therefore does not have any myofibrillar tissue impediment for its contraction.

3.2. Physical properties of the enzymatically pretreated samples

First, the enzyme incorporated into the samples was evaluated by assuming that the changes in weight that took place during the enzyme pretreatment were all caused by enzyme solution intake. For the I pretreatment, the samples that had the greatest weight increase were the unaged ones (0A_I = 4 ± 0.6%; 7A_I = 3.1 ± 1%). For VI, were the aged (0A_VI = 2.7 ± 1.3%; 7A_VI = 4.3 ± 1.7%). The greater structural integrity of the unaged samples (0A) better retained the injected solution but difficult the impregnation when they were VI. The opposite occurred with the aged samples (7A).

The statistical analysis of the hardness data of the pretreated samples after cooking was evaluated in line with the results observed for the C samples. That is to say, with the recoded of the samples according to batches aging dependency observed for control. Therefore, samples were re-coded from 4 (0_I, 7_I, 0_VI and 7_VI) to 8 (0_I_AEF, 0_I_NAEF, 7_I_AEF, 7_I_NAEF, 0_VI_AEF, 0_VI_NAEF, 7_VI_AEF, and 7_VI_NAEF). Fig. 3 presents the hardness results of the samples pretreated with the enzyme by I or VI per heat treatment. The results showed the strong enzyme effect. Although cooking increased the hardness of the C samples (Fig. 2A), regardless of aging, the pretreatment with the enzyme reduced it for them all except the AEF samples pretreated and cooked at 80 °C for 10 min (Fig. 3). Papain is a highly efficient enzyme that leads to the significant degradation of both myofibrillar and collagen proteins (Ashie, Sorensen, & Nielsen, 2002) by specificity action on amino acids with aromatic side chains, such as Phe (Phenylalanine) and Tyr (Tyrosine), in the P2 position (Singh et al., 2018). By comparing the two methods to apply the enzyme, without samples cooked at 80 °C for 10 min, VI minimized the aging effect ($P = .06$), cooking treatment ($P = .87$) and re-coded according to the C classification (AEF or NAEF) ($P = .14$). Instead cooking conditions and the C classification were significant ($P < .001$) when I was employed. For this pretreatment, all the samples' hardness, independent of the baches (AEF and ANEF), increased with a rising temperature and longer cooking times, even though the samples of AEF batches were the hardest under all the cooking conditions. This result could be related to enzyme dispersion inside samples and a short action time. With the VI technique, the enzyme was incorporated and dispersed inside the sample. With the I method, it was only located at the injection points, generating its action only in them because did not have time to diffuse through the sample because it was cooking at the same time. This result indicates marked papain enzyme activity at the applied temperatures and times which did not only minimize the increase in the C samples' hardness caused by cooking but also made them softer, except for treatment at 80 °C for 10 min. Therefore, the increasing temperature during cooking suffices to cook meat and accelerates enzyme activity until meat is tenderized. Several authors have reported

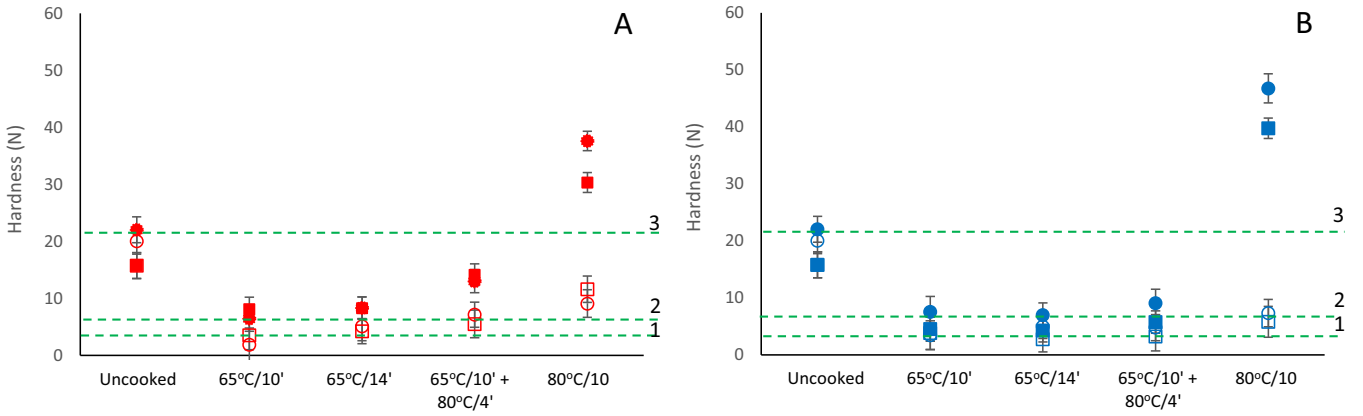

Fig. 3. Means ± standard error of hardness values for the samples pretreated with enzyme by injection (_I) (A) or vacuum impregnation (_VI) (B) at each heat treatment. Filled symbols: batches of samples whose values differed depending on aging (AEF). Empty symbols: batches of samples whose values were equal independently on aging (NAEF). Dots: aging time = 0 (0A). Squares = 7 aging days (7A). Green dashed lines: extrusion force levels for dysphagic people: 1 = soft (4.1 N); 2 = medium (7.8 N); 3 = hard (23.5 N) (Ibañez et al., 2019). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the softening effect of papain, although the results obtained by each one were highly dependent on the type of meat used, the concentration of enzyme applied, the application method used, the cooking conditions and the texture measurement method used. Barekat & Soltanizadeh, 2017 and Botinestean et al., 2017, working with beef observed a hardness reduction of 19 and 15% respectively. Ma et al., 2019, employing Yak meat, observed a hardness reduction with the increases in the cooking time. Cooking at 50 °C, they observed the lowest hardness at 120 min (49% of hardness reduction) although it increased with 150 min (only 18% of hardness reduction). In the present study, samples 0A_VI_AEF, cooked at 65 °C for 10 min, had a hardness reduction of 65.9% although an increase of 53% when cooked at 80 °C for 10 min. Samples 0A_VI_NAEF, at the same cooking condition, had a hardness reduction of 82.7% and 73.6% respectively. So, employing VI, except for the cooking condition of 80 °C for 10 min, and I, except for the two highest cooking conditions, processed meat could be classified as soft or medium according to the classification done by Ibañez, Gómez, Merino, & Beriain, 2019. Those authors classified foods for dysphagic people at three extrusion force levels: soft (4.1 N); medium (7.8 N); hard (23.5 N) (green dashed lines in Fig. 3).

Enzyme action brought about meat structure changes that strongly affected sample volume and color during cooking. These changes were the same regardless of the sample batches labeled according to the C classification (AEF or NAEF), and even for those cooked at 80 °C for 10 min. The data of both were processed together. Fig. 4A and Fig. 4B respectively depict the area variation (%) and the redness index (a*/b*) for the unaged or aged samples pretreated by both methods and cooked under each heat condition. For each pretreatment, both cooking and aging were significant ($P < .001$). For the unaged samples (vacuum impregnated), a slight area increase was noted for the lowest intensity cooking conditions, and was constant for the I ones. Area reduction occurred in both samples under the highest cooking condition, but this reduction was more marked for the impregnated ones. The result agrees with hardness (Fig. 3) and could also express how impregnation is the better of the two ways to apply enzymes. The greater enzyme dispersion in samples more significantly destroyed the myofibrillar structure (structural relaxation) by increasing the area under low intensity cooking conditions. At high intensity (80 °C for 10 min), as mentioned above, shrinkage could occur because of collagen contraction, which could be greater for the impregnated samples because the enzyme eliminated the physical impediment that myofibrillar tissue posed. This could be why the shrinkage of the aged samples was greater. By way of example, Fig. 5 shows some samples. We can see how the samples cooked at 80 °C for 10 min had the smallest area, which was slightly bigger for the unaged samples that were impregned and cooked for 10 min at 65 °C (65 °C - 10′).

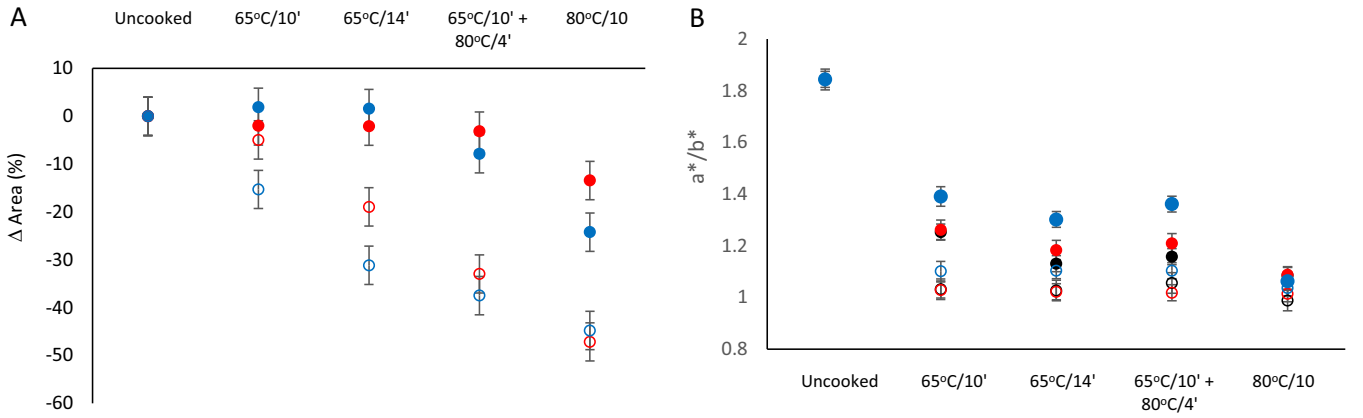

Fig. 4. Means ± standard error of area changes (%) (A) and redness (a*/b*) (B) at each heat treatment. Black dots: control samples. Red dots: samples injected with the enzyme. Blue dots: samples vacuum impregnated with the enzyme. Fillet dots: aging time = 0 (0A). Empty dots = 7 aging days (7A). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

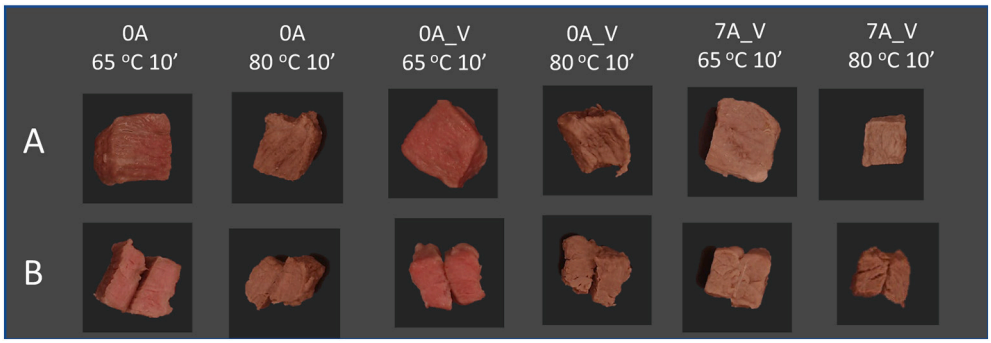

Fig. 5. Example of the samples used in the study. A: entire sample. B: cut sample. 0A - 7A = aged for 0 or 7 days; _VI = vacuum impregnated; 10′ = 10 cooking minutes.

Regardless of the pretreatment used, cooking intensity decreased samples' lightness (values not shown) and redness ($P < .05$) (Fig. 4B) from red to brown/Gy. For both pretreatment, values of lightness were lower for unaged samples ($P < .05$), being higher for redness ($P < .001$). Change in lightness is caused by myosin denaturation, which starts at about 35 °C (Pakula & Stamminger, 2012). As temperature increased, more pigments were denatured, meat color changed to brown/Gy and pigments were responsible for the brown/Gy color: denatured globin nicotinamide hemichromes, denatured myoglobin, Maillard reaction products, metmyochoromogen and/or haematin diimadazole complexes (Xia, Weaver, Gerrard, & Yao, 2008). Redness decrease was also observed by other authors (Botinestean et al., 2018). The changes in color of the impregnated unaged samples cooked under lower conditions were not the same, and a higher reddish coloration appeared that conferred it a slightly raw appearance (Fig. 5A and B). Myoglobin redox chemistry is the primary determinant of cooked meat color. Higher deoxymyoglobin levels before cooking maintain a slight post-cooking pink color, but brown prematurely appears if oxy- and metmyoglobin are present (Mancini & Hunt, 2005).

3.3. Sensory analysis

The sensory analysis was done after studying the factors batch, aging, enzyme pretreatment and cooking process. To reduce the number of samples tested by the panelists, softness from texture analysis was the main criterion applied to select them. As impregnation minimizes all the factors (batch, aging and cooking condition), it was selected as a pretreatment and the cooking condition generated the softest samples 65 °C for 10. So the first type of selected samples were those unaged, pretreated with VI and cooked at 65 °C for 10 min (0A_VI at 65 °C 10′). For the C samples, the same sample type and cooking conditions were selected (0A at 65 °C 10′). To evaluate the highest redness color values of samples 0A_VI at 65 °C 10′, samples 0A_VI at 80 °C 10′ and 7A_VI at 65 °C 10′ were also selected (with lower redness values), even though their area was smaller.

To compare the hardness values of the samples rated by the panelists, some of them (from each treatment) were also analyzed by the compression test. Table 1 presents the mean values and error standards of the compression test, and of the sensory analysis of both meat and dish (meat in soup). All the samples' hardness values fell within their processing range and equalled the previously evaluated ones. For hardness and redness, panelists' answers agreed with those obtained by the instrumental analysis. The control samples were evaluated as the hardest ($P < .001$), while the rest showed no differences from one another. Sullivan & Calkins, 2010 reported a significant increase in tenderness for treated samples observed by panelists. Employing an 8-point scale from less to high, control samples were evaluated as 5 and 5.9 the treated. In the present study, using a 9-point hedonic scale (9 = very much hard and 1 = very much soft), control samples were evaluated as 7.1 while treated from 4 to 3.4 (Table 1). For redness, control and 0A_VI (65 °C 10′) were the reddest with a statistical difference ($P < .001$) with 0A_VI (80 °C 10′) and 7A_VI (65 °C 10′). This result is illustrated in Fig. 5 and Fig. 4B, obtained by the image analysis. Perhaps it was because of their highest redness values that the panelists evaluated them as the samples that looked less well-done ($P < .001$). However, samples' overall appreciation was statistically the same for all the samples cooked at 65 °C for 10 min. Like the C samples, they all scored higher than the mean value of 4.5. Only the samples cooked at 80 °C for 10 min scored lower than the mean ($P < .05$). Perhaps their overcooking, which gave them a dry sample appearance, was behind their low score. As it is well-known, increased cooking intensity generates more cooking loss (Barbanti & Pasquini, 2005; Wood, Nute, Fursey, & Cuthbertson, 1995), which could become more remarkable because of enzyme action.

For overall dish acceptance, samples 0A_VI (65 °C 10′) obtained the lowest values, even below the mean scale value and despite there being no statistical differences between treatments ($P = .52$). This result could be due to the high redness value that made samples look raw (Fig. 5).

According to the physico-chemical and sensory results, the best process to obtain the softest meat without disturbing its original shape for meat or meat on a dish would be to use aged meat, and pretreatment

Table 1
Means values and error standards of the compression test (N), sensory analysis of meat and meat on a dish (meat in soup).

	Compression test	Sensory analysis of meat				Sensory analysis of mean on dish
	Hardness (N)	Hardness	Redness	Doneness appearance	Overall acceptance	Overall acceptance
0A 65 °C 10'	42.9 ± 6.8a	7.1 ± 0.5a	6.3 ± 0.4a	4.3 ± 0.5a	6.5 ± 0.5a	5.0 ± 0.6a
0A_VI 65 °C 10'	8.5 ± 7.6b	3.4 ± 0.5b	6.5 ± 0.4a	4.2 ± 0.5a	5.3 ± 0.5a	3.9 ± 0.7a
0A_VI 80 °C 10'	8.3 ± 6.8b	4.0 ± 0.5b	4.1 ± 0.5b	6.7 ± 0.5b	3.8 ± 0.5b	4.5 ± 0.6a
7A_VI 65 °C 10'	7.6 ± 7.6b	3.7 ± 0.5b	3.2 ± 0.4b	6.5 ± 0.5b	5.7 ± 0.5a	4.3 ± 0.7a

Different superscripts in the same column indicate significant differences among samples ($p < .05$).
0A - 7A = aged for 0 or 7 days; _VI = vacuum impregnated; 10′ = 10 cooking minutes.

with enzyme by VI and cooked at 65 °C for 10 min.

4. Conclusion

Both I and VI were evaluated to know which was the better method to apply papain to obtain the highest softness meat values without disturbing its original shape and cooking conditions. By means of both, postcooked meat was classified as soft (4.1 N) or medium (7.8 N) in almost all cases, which makes it suitable for people with poor oral health as in dysphagia. From the meat-tenderizing point of view, VI was presented as the better enzyme pretreatment method because it minimizes important factors linked with the type of muscle from which meat comes, aging and cooking conditions. However, it generates reddish samples that look like raw meat, and were rejected by the panelists, even if the problem was minimized when aged meat was used. The tested cooking conditions were enough to cook meat and to accelerate enzyme activity until meat was tenderized. So the best meat processing to obtain the highest softness and best appreciation values by panelists for both meat alone and meat on a dish is to use aged meat pretreated by VI and cooked at 65 °C for 10 min. New studies are necessary to evaluate the obtained meat's nutritional quality.

CRediT authorship contribution statement

Raúl Grau: Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Supervision, Project administration, Funding acquisition. **Sergio Hernández:** Conceptualization, Methodology, Investigation, Visualization, Writing – original draft. **Samuel Verdú:** Investigation, Visualization, Writing – original draft. **José M. Barat:** Writing – review & editing. **Pau Talens:** Conceptualization, Methodology, Investigation, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

None.

Data availability

No data was used for the research described in the article.

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