Research article

Quantitative descriptive analysis as a strategic tool in research activities relating to innovative meat tenderisation technologies

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A B S T R A C T

Sensory analysis plays a significant role in developing innovative technology from prototype to industrial stage, and above all, in the meat industry.

The starting hypothesis is that the quantitative descriptive analysis is crucial to optimise an innovative process for tenderising meat before the scale-up stage because it provides information that instrumental and consumer science analyses cannot achieve. With this in mind, the present study describes the detailed protocol of the quantitative descriptive analysis, which was developed and optimised to contribute to the prototype development stage of new meat tenderising technology.

This study applied the quantitative descriptive analysis to evaluate the sensory characteristics of *semitendinosus* beef meats submitted to the tenderising process by combining exogenous enzymes and ultra-sound radiation treatments. A correlation analysis was performed among sensory and instrumental data. A significant and negative correlation was found only among texture parameters evaluated by sensory and instrumental parameters ($R > -0.81$ and $P < 0.05$). Conversely, no significant correlation ($P > 0.05$) was found between sensory and instrumental chromatic characteristics. Moreover, the quantitative descriptive analysis was a valuable tool because it provided precious information on the appearance of the treated raw meat (score less than 6), which was not detected by instrumental analyses. This information is precious because the appearance of raw meat is fundamental to the consumer buying decision process. Based on the results obtained through sensory analysis, we could highlight the necessity of optimising technological processing before the industrialisation stage to avoid a probable failure of this production method when applied to the market.

1. Introduction

Technological innovations in the food industry must be realised in different stages. Achieving a successful innovation process requires a critical and accurate analysis of the results obtained at each stage before proceeding to the next step. In the innovation process, prototype realisation is fundamental [1], which affects the timing and success or failure of the industrialisation and commercialisation stages. Therefore, having an overall vision of the food is crucial, considering its chemical, physical, microbiological and sensory characteristics. The lack of sensory testing was unanimously listed as the biggest challenge in the prototyping stage. Preference and discrimination tests are the most commonly used in the food industry to test if a product differs from another [2].

Regarding the meat industry, the proper application of sensory analysis and the elaboration of data play a fundamental role in developing innovative methods to obtain cheap and environmentally friendly products since this method supplies helpful information to all the players in the supply chain. New sensory techniques relying on fast, accurate, informative and intuitive descriptive methods have also been employed to characterise traditional and new meat products. The techniques are napping, flash profile [3], check-all-that-apply [4] and rate-all-that-apply methods [5]. Other innovative sensory methodologies aim to approach more realistic assessment models by analysing how sensations vary over time. Among these temporal sensory techniques, time intensity [6] and temporal dominance of sensations [7] are of particular scientific and technological interest.

However, meat scientists have generally not taken advantage of these innovative approaches because of some drawbacks and limits in their application to samples that need processing (e.g. cooking or roasting) before tasting by the panellists and the lack of accurate results if compared to conventional descriptive techniques [8].

The classic and most used sensory method in meat science is quantitative descriptive analysis (QDA), which can provide the most significant amount of information and is easily interpreted in the elaboration of new products [9], even if it requires considerably more time and costs to realise compared to new sensory tests. These drawbacks and the absence of a specific and standardised protocol for the QDA method applied to meat products, unlike other foods and drinks such as wine and oil, make its application difficult and poor.

The present research aims to address these gaps by providing a detailed and correct protocol for applying QDA to meat products. Moreover, this study hypothesises that the quantitative descriptive analysis is crucial to optimise an innovative process for tenderising meat before the scale-up stage, highlighting QDA's contribution to the prototypal development. The proposed methodology is based on many sessions, and it considers the global and individual panellist performance in terms of discriminability, repeatability, exactness/inefficiency (the judge effect) and agreement/disagreement with the panel (the interaction of the judge with the evaluated product).

The innovative technological process considered in this study is that proposed by Marino et al. [10], which aimed to replace the conventional method based on refrigerating storage (3 °C) with a combination of enzymatic (papain) and physical methods (ultra-sound radiation). Textural profile and proteomic results suggested that ultra-sound applied before papain treatment can modulate the tenderisation rate with a slower hydrolysing degree, favouring the preservation of the meat structure regarding chewiness and myofibrillar protein degradation. In addition, De Devitiis et al. [11] reported that consumer purchase intention for meat treated by the Marino et al. [10] method was strongly influenced by perceived benefits (trust in science) and weakly influenced by perceived risks. Consequently, the concordance between results obtained through the proteomic approach of Marino et al. [10] and the correct sensory analysis evaluation (QDA method) application in this study was investigated.

2. Materials and methods

2.1. Experimental design and treatment application

The experiment used a completely randomised design with a factorial arrangement. Each treatment was replicated four times to ensure reliability and reduce the impact of variability. The treatment combinations consisted of two meat tenderising technologies: storage at 3 °C (a conventional method considered a control and named as *untreated* samples) and a combination of ultra-sound
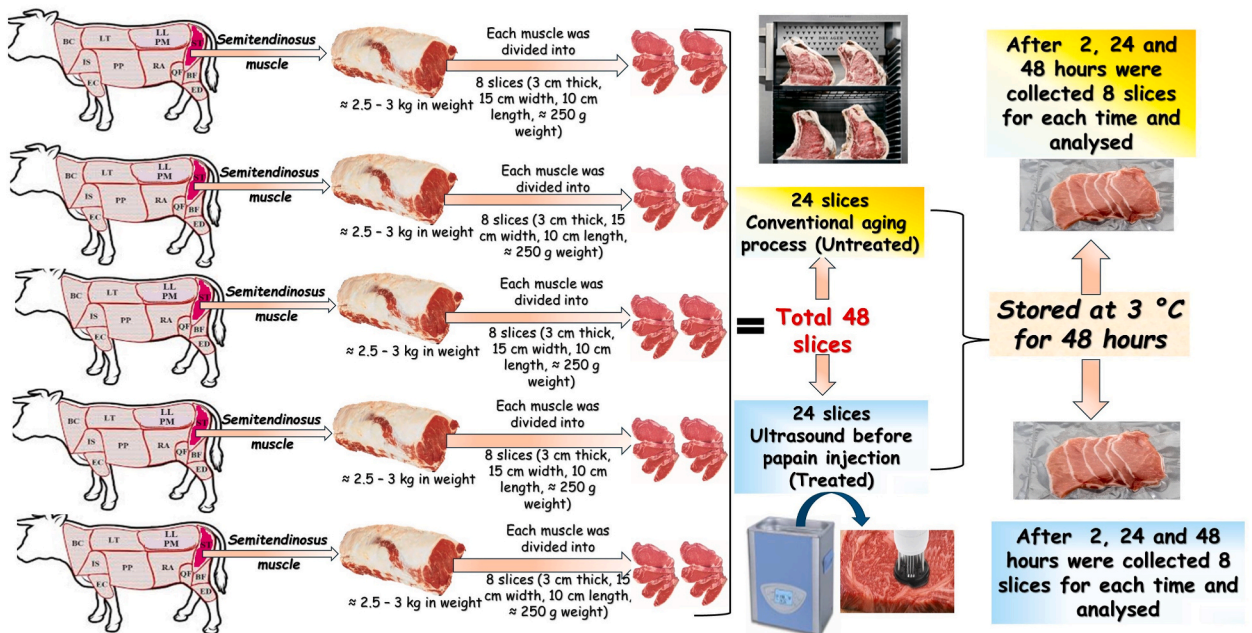

Fig. 1. Diagram of the experimental plan.

radiation and papain injection (an innovative method named as *treated* samples). Randomisation was performed for each replicate independently [12]. This randomisation process ensured that treatments were randomly assigned, minimising potential errors or confounding factors that could have affected the results. Samples stored at 3 °C were collected from each treatment at 2, 24 and 48 h for analysis. Fig. 1 shows a detailed diagram of the experimental plan used in this study.

2.2. Meat sample collection and preparation

Five *semitendinosus* muscles (approximately 2.5–3 kg in weight) collected from five male beef cattle were purchased from a local slaughterhouse. All animals were reared on the same farm and slaughtered at approximately 18 months old, following industrial routines used in Italy according to EU rule n. 1099/2009. Carcasses were chilled at 2 °C–4 °C for 24 h according to standard commercial practices. After 24 h post-mortem, each muscle was removed from the left and right sides of each cold carcass (2 °C–4 °C) and transported to the laboratory under refrigerated conditions (at a maximum temperature of 4 °C). Surface fat, silver skin and external connective tissues of the *semitendinosus* muscles were carefully removed with a sharp knife. Each muscle was divided into eight uniform slices, obtaining a total of 48 pieces, which were randomly allocated into two experimental groups with different tenderisation treatments. Meat samples were exposed first to ultra-sound treatment and, after injection of papain solution by multi-needle syringe, to the same operating conditions and with the same devices reported by Marino et al. [10]. A flexible film (polyamides 20 μm thick coupled with polyethylene 70 μm thick, oxygen transmission rate of less than 50 cm^3/m^2·at 0.1 MPa·for 24 h) was used during storage to pack all sample meat.

2.3. Analyses

The evaluation of chromatic characteristics and image analysis were performed on raw meat. Mechanical properties and sensory analysis (except descriptors of colour and appearance) were done on cooked meat. All determinations were replicated five times at least.

2.3.1. Sensory analysis

The sensory characteristics of meat were evaluated through the constitution of a panel of selected and trained judges to objectively describe, define and discriminate the sensory properties of beef treated with conventional and innovative tenderising methods. The tests were performed in the sensory laboratory of the DAFNE Department of the University of Foggia (Italy), designed according to ISO 8589 [13].

The trained sensory panel was formed through *recruitment*, *selection* and *training* stages. The choice of subjects and the number of panellists were conducted according to ISO standards 8586-1 [14] and 11035 [15]. Fig. 2 outlines the protocol used to form the group of meat expert tasters. Detailed information on protocol analysis was reported in the supplementary contents (Annex I).

The meat samples used for taste were cut into many cube shapes (2 × 2 × 2 cm) and presented to tasters in three coded cups (each cup contained three parts) and cooked through an electrical grill at 250 °C until the internal temperature of the meat pieces reached
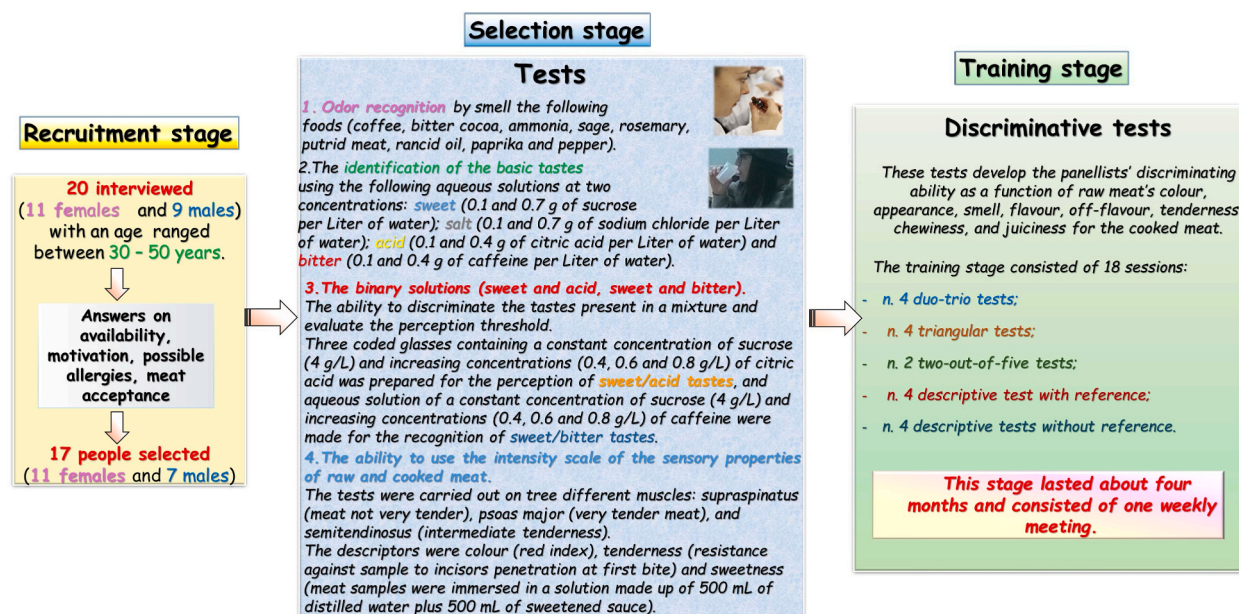

Fig. 2. Protocol used to form the group of meat expert tasters.

70 °C.

The trained panellists conducted QDA on the tenderised meat using innovative and conventional methods. Each judge performed all tests in a single cabin using white light. Each sample was served in three-digit-coded aluminium trays (250 ml, $128 \times 100 \times 32$ mm) at approximately 50 °C.

2.3.2. Chromatic characteristics

Colour parameters were determined by a colourimeter model Konica Minolta CR-400 (Konica MINOLTA, Osaka, Japan) equipped with D65 (6540 K clear daylight) as the light source, a standard observer of 2° and an aperture size of 8 mm. colour was expressed in terms of $L*$ (Luminosity), $b*$ (yellow index) and $a*$ (red index) according to the standard conditions of the *Commission International d'Éclairage* (CIE). Each sample was left at 4 °C for 2 h (blooming time) before the colour evaluation.

The calculations were expressed as the hue angle (H °) calculated as arctg (b*/a*) and chroma (C) calculated using Eq. (1).

$$C = \sqrt{a^2 + b^2} \tag{1}$$

2.3.3. Mechanical properties

Cooked meat was submitted to Warner-Bratzler shear force (WBSF) and texture profile analysis (TPA). Steak samples (2.0 cm of thickness) were grill-cooked at 250 °C until the core of pieces reached 80 °C. Ten blocks with sides at right angles and a 1 cm^2 cross-sectional area cut parallel to the muscle fibre direction were obtained for each sample. An Instron 3343 universal testing machine (Instron Ltd., High Wycombe, UK) was used for both instrumental tests. The shear force was measured considering the peak force (N) required to cut the meat block in two halves perpendicular to its length through a Warner-Bratzler device. The steak samples were sheared perpendicular to the fibres at a 100 mm/min crosshead speed through a 1000 N load cell. Results consisted of recording the peak force produced during analysis for each piece. The TPA was done using a compression device that avoided transversal elongation of the samples. Each sample underwent two cycles of 80 % compression.

2.3.4. Macro-structure evaluation by image analysis

The macro-structure of raw meat was observed under a stereomicroscope model SMZ-1 (Nikon Instruments, Sesto Fiorentino, Florence, Italy) at a magnification of $\times$ 32 for 30 min. The samples were sectioned by bistoury to obtain slices with a parallelepiped shape ($2 \times 2 \times 0.5$ cm) and placed on a clean microscope slide. Twenty sections (five replications for four treatments, including control) were examined immediately after each technological treatment. The four treatments included the conventional ageing method, the application of ultra-sound sonication only, the injection of a papain solution and the combination of ultra-sound radiation followed by the papain solution (see paragraph 2.1). The images of the meat sections were acquired using a digital camera, the Nikon (Netherlands) Digital Sight 10, equipped with a colour CMOS image sensor (1/2.8 inch), which is recordable at 1920×1080 pixels.

2.4. Data analysis

2.4.1. Statistical elaboration of sensory analysis results

The data elaboration of the selection stage involves the *z-test* and *sequential analysis*.

The performance of the aspiring panellists at the end of the selection stage was evaluated by calculating the z estimator by comparing the average score of all aspiring judges (obtained during tests of odour, taste and intensity scales of meat attributes) with that of the single candidate. The calculation was made using Eq. (2).

$$z = \frac{Average\ of\ Candidate\ -\ General\ Average}{Standard\ Deviation} \tag{2}$$

A value of Z $\geq$ 0 indicates that the average of the aspiring panellist was more than or equal to the group average, while a value of Z < 0 suggests that it was lower than the group average.

The *sequential analysis* applied to the data collected during the panellists' training stage consisted of plotting the total number of correct responses as a function of the number of trials to obtain a graph with two parallel straight lines, L_0 and L_1, which identified three regions: acceptance, test continuation and rejection. The correct answers of the first test were entered as (x, y) = (1, 1), while the wrong results were reported as (x, y) = (1, 0). In the following tests, x and y were increased by 1 for each correct answer and by 0 for each wrong result. The tests were conducted until the related points intersected one of the lines, limiting the indecision region. The aspiring panellists were accepted or rejected as a function of the results associated with L_0 and L_1. The L_0 and L_1 lines were drawn as a function of $p_0 = 0.65$, $p_1 = 0.90$, $\alpha = 0.025$ and $\beta = 0.025$. The letter α represented the probability of rejecting an acceptable candidate, while the letter β represented the probability of selecting an unacceptable one. The parameter p_0 represented the maximum unacceptable ability, and p_1 represented the minimum adequate ability (both parameters were determined as the proportion of correct responses). The graph was divided into regions as a function of the values chosen for α, β, p_0 and p_1. The relations that computed the slopes and intercepts of straight lines were calculated as follows (Eqs. (3)–(5)):

$$\beta = \frac{k_2}{k_1 - k_2}; \ \alpha_0 = \frac{e_1}{k_1 - k_2}; \ \alpha_1 = \frac{e_2}{k_1 - k_2} \tag{3}$$

Where:

$$k_1 = \log p_1 - \log p_0 \; ; \; k_2 = \log(1-p_1) - \log(1-p_0) = \log q_1 - \log q_0 \tag{4}$$

$$e_1 = \log \beta - \log(1-\alpha); \; e_2 = \log(1-\beta) - \log \alpha \tag{5}$$

The global and individual panellists' ability to discriminate, repeat and give exact or wrong answers (the Judge effect) and the alignment of each taster with the panel (the interaction of the judge with the evaluated product) were evaluated by the Student t-test. The mean values of the scores that each panellist attributed to each meat sensory characteristic during the six training sessions (one for each of the 6 weeks) were compared to those provided by the whole panel.

The Student t-test was used to reject the hypothesis that the group's means were identical and that differences between them, whether observed, reflect a discrimination between the sample populations of the two classes. A probability (P-value) was estimated under the null-difference hypothesis for each sensory attribute of meat. In this case, if the Student t-test obtained a P-value less than 0.05, it means that the average scores given by each panellist during the six sessions (n. six repetitions) for each sensory attribute differed significantly from the average scores given by the whole panel (n. 72, i.e. 6 repetitions × 12 panellists). In this case, the evaluation of the single panellist was considered in disagreement with that of the whole panel and thus unreliable.

2.4.2. Statistical methods used to elaborate sensory and instrumental data obtained from meat samples analysed

Sensory (QDA descriptors) and instrumental data (L*, hue angle, chroma, Warner Bratzler Shear Force (WBSF) and mechanical parameters of texture profile analysis (TPA)) were statistically analysed by a two-way ANOVA using the general linear model procedure of the StatSoft software, version 6.0 (StatSoft, OK, USA). Fixed effects of the meat tenderising technological treatment and storage time at 3 °C were included in the following model (Eq. (6)):

$$Yij = \mu + Bi + Fj + (BxF)ij + \varepsilon ij \tag{6}$$

where Yij is the observation of each dependent variable, μ is the overall mean, Bi is the effect of the tenderising technological treatment, Fj is the effect of the storage time, (BxF)ij is the interaction between the tenderising technological treatment and the storage time and εij is the residual random error associated with the observation. The mean values were compared using Fisher's LSD test to estimate the significant differences between means (P < 0.05). The interaction interactive term was initially included in the model, but successively, it was not included in the results table due to the absence of statistical significance (P > 0.05) for any of the evaluated variables.

The correlations between sensory (QDA descriptors) and instrumental (L*, hue angle, chroma, WBSF and mechanical parameters of TPA) data referring to the meat submitted to two tenderising methods and stored for 48 h were determined using Pearson's linear correlation coefficient. The correlation coefficients higher than 0.8 (P < 0.05) were considered significant.
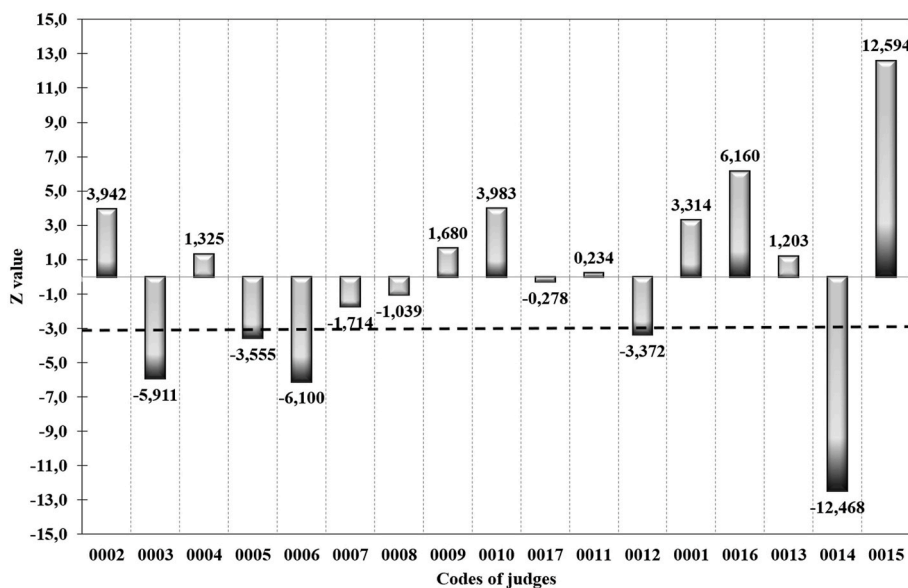

Fig. 3. Values of the Z parameter calculated for each candidate as a function of the results obtained during the selection tests.

3. Results and discussion

3.1. Sensory analysis

3.1.1. Global performance at the selection stage

Fig. 3 shows the values of the z parameter calculated for each candidate as a function of the results obtained during the selection tests. Nine candidates out of 17 had scores higher than 0 and were selected for the tasting group. Five tasters (coded **0003**, **0005**, **0006**, **0012** and **0014**) showed the lowest Z values (<-3) due to a poor ability to perceive fundamental tastes even at extremely high concentrations. Therefore, they were discarded from the sensory panel. However, three candidates (coded **0007**, **0008** and **0017**) were selected for the training stage, even if they had a Z score lower than zero but higher than -3. This decision was made since the Z value lower than zero was due to errors made by these tasters in a single test, and, in any case, they were not wrong in the assessment of the intensity scales test applied to the meat.

3.1.2. Ability to discriminate meat samples and training stage

The selection of tasters continued through discriminant tests (duo–trio, triangular and two out of five tests), and the results were elaborated on by sequential analysis. Fig. 4 shows that all testers had a score above the acceptance line or coincident with it ($L_1 = 2.107 + 0.505 \times n$) after the seventh test; therefore, they were considered eligible for training within the applied statistics. Moreover, it is possible to observe that in the second stage of the discriminating tests (from the eighth to the final stages), all candidates obtained a higher score than the acceptance line ($L_1 = 2.107 + 0.505 \times n$).

The training process for the 12 selected judges was conducted through six descriptive tests with and without references. The cuts of meat evaluated by the tasters during the training stage with the descriptive tests were the following: *supraspinatus* muscle (meat not very tender), *psoas major* muscle (very tender meat) and *semitendinosus muscle* (used as a reference and having an intermediate degree of tenderness) coming from bullock. The intensity scales for each attribute ranged from 0 to 10, and the descriptors considered were appearance, colour, odour, off-flavour, taste, tenderness, chewiness, juiciness and global score. These tests aimed to evaluate the ability of each taster to attribute the same score to the same type of meat evaluated in different sessions and conducted one week apart. Moreover, to verify the homogeneity of the sensory panel, the average ratings of each taster obtained in the different tasting sessions were compared to the average values of the ratings given for each descriptor by the tasting group using the Student t-test. Results demonstrated that almost all tasters gave answers similar to the panel groups' for each evaluated meat sample descriptor (Tables 1 and 2). In fact, the mean values of each tester for each descriptor were not significantly different from the average values of the sensory panel in all sensory sessions. The only exception found for evaluating the fillet beef colour was by taster coded **0017**, who attributed a lower score than the average sensory panel (3 vs. 6.37) in all the sessions on this type of meat. Moreover, tasters coded **0002** and **0007** distinguished their evaluation of odour for fillet beef from that of the average sensory panel, thanks to their lower standard deviations (Table 2). These results highlight the reliability and repeatability of the tasters' judgements in describing the different types of meat in the various tests conducted at different times and with cuts of beef having different sensory characteristics, confirming the homogeneity of evaluation in the description of the meat by panel group, with the elimination of subjective evaluations linked to the history and experience of each taster, making it a valid analytical tool in terms of reliability, repeatability, precision and accuracy. Further confirmation of the validity and homogeneity of the selected and trained group of tasters is given in Fig. 5, which shows the QDA profiles of meat samples evaluated during the training stages. In fact, all judges appreciated the *psoas major* (very tender meat) better
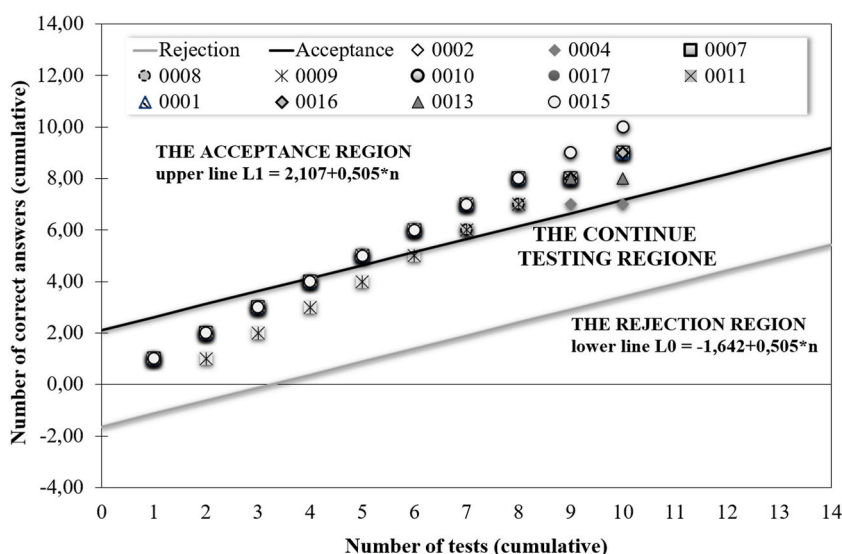

Fig. 4. Sequential analysis applied to discriminating tests to select and train tasters with $p_0 = 0.33$, $p1 = 0.66$, $\alpha = 0.05$ and $\beta = 0.10$.

Table 1

Sensory descriptive tests of the ***supraspinatus* muscle** carried out during the training step: results of the Student t-test applied to the mean values assigned by each taster compared with the mean values of the whole panel.

	Panel	0002[b]	0004	0007	0008	0009	0010	0017	0011	0001	0016	0013	0015
APPEARANCE													
Mean	5.0	4.7	5.2	5.3	3.7	5.4	7.0	4.9	5.0	4.5	5.2	3.7	4.8
Dev st.	0.79	0.58	1.46	1.57	1.15	2.58	1.83	2.32	1.00	1.36	0.35	1.25	0.29
SEM[a]	0.23	0.33	0.84	0.91	0.66	1.49	1.06	1.34	0.58	0.79	0.20	0.72	0.17
t-value		−0.5	0.3	0.3	−1.6	0.3	1.8	0.0	0.1	−0.5	0.5	−1.4	−0.3
F-ratio variances		1.9	3.5	4.0	2.2	10.7	5.4	8.7	1.6	3.0	5.2	2.5	7.4
COLOUR													
Mean	5.3	5.3	5.9	4.7	5.0	5.7	6.1	4.8	5.0	5.2	4.8	5.7	4.8
Dev st.	0.26	1.53	0.90	0.61	2.00	2.04	1.71	2.14	1.00	0.68	0.35	1.13	0.35
SEM	0.08	0.88	0.52	0.35	1.15	1.18	0.99	1.24	0.58	0.39	0.20	0.65	0.20
t-value		0.1	1.2	−1.5	−0.2	0.4	0.9	−0.4	−0.4	−0.1	−1.8	0.7	−1.8
F-ratio variances		34.9	12.1	5.5	59.8	62.3	43.7	68.2	14.9	6.9	1.8	19.0	1.8
ODOUR													
Mean	5.5	5.0	6.0	4.0	5.7	7.4	7.0	3.4	5.0	4.73	5.3	7.2	5.3
Dev st.	0.68	0.06	1.05	1.70	1.10	2.18	1.95	2.23	1.00	1.62	0.49	1.88	0.26
SEM	0.20	0.03	0.61	0.98	0.64	1.26	1.13	1.29	0.58	0.94	0.28	1.09	0.15
t-value		−1.2	0.7	−1.4	0.3	1.5	1.2	−1.5	−0.7	−0.8	−0.4	1.4	−0.5
F-ratio variances		137.44	2.41	6.34	2.65	10.35	8.30	10.83	2.18	5.70	1.88	7.73	6.54
OFF FLAVOUR													
Mean	0.3	0.3	0.0	0.0	0.0	0.8	0.0	1.5	0.0	0.6	0.0	0.0	0,0
Dev st.	0.25	0.58	0.00	0.00	0.00	1.44	0.00	1.33	0.00	0.87	0.00	0.00	0,00
SEM	0.07	0.33	0.00	0.00	0.00	0.83	0.00	0.77	0.00	0.50	0.00	0.00	0.00
t-value		0.2	−1.9	−1.9	−1.9	0.7	−1.9	1.6	−1.9	0.6	−1.9	−1.9	−1.9
F-ratio variances		5.29	0.00	0.00	0.00	33.08	0.00	28.15	0.00	12.07	0.00	0.00	0.00
TENDERNESS													
Mean	3.7	3.0	3.7	3.6	3.7	2.5	5.0	2.6	4.7	4.4	3.7	3.6	3,5
Dev st.	0.88	2.00	1.13	0.64	1.15	2.50	0.95	1.12	0.58	1.04	1.61	1.58	1,86
SEM	0.25	1.15	0.65	0.37	0.66	1.44	0.55	0.65	0.33	0.60	0.93	0.91	1.07
t-value		−0.5	0.0	−0.1	0.0	−0.8	1.8	−1.3	1.6	0.9	0.0	0.0	−0.1
F-ratio variances		5.13	1.63	1.93	1.71	8.01	1.16	1.59	2.34	1.38	3.32	3.21	4.43
CHEWINESS													
Mean	3.6	3.0	3.2	3.6	3.6	2.6	4.7	2.8	4.7	4.3	3.7	3.1	3,3
Dev st.	1.08	3.05	1.71	0.64	1.58	2.51	1.42	0.98	0.58	1.45	1.53	1.81	1,53
SEM	0.31	1.76	0.99	0.37	0.91	1.45	0.82	0.57	0.33	0.84	0.88	1.05	0.88
t-value		−0.28	−0.30	0.11	0.07	−0.58	1.15	−0.94	1.57	0.68	0.10	−0.37	−0.21
F-ratio variances		8.02	2.52	2.88	2.16	5.44	1.74	1.20	3.48	1.80	2.01	2.82	2.01
JUICINESS													
Mean	4.4	3.7	3.2	4.3	4.0	4.0	5.4	3.1	5.3	5.1	5.0	4.0	5,5
Dev st.	0.75	1.46	1.71	1.53	1.00	3.65	1.73	1.16	1.53	1.50	1.00	2.46	0,50
SEM	0.22	0.84	0.99	0.88	0.58	2.11	1.00	0.67	0.88	0.87	0.58	1.42	0.29
t-value		−0.8	−1.1	−0.1	−0.5	−0.2	0.9	−1.6	1.0	0.7	0.8	−0.3	2.1
F-ratio variances		3.9	5.2	4.2	1.8	23.9	5.4	2.4	4.2	4.1	1.8	10.9	2.2
TASTE													
Mean	4.7	4.0	3.4	3.7	4.3	3.3	6.3	4.1	4.7	6.0	5.4	4.5	6,1
Dev st.	0.47	1.73	1.44	0.58	1.15	2.89	1.53	0.82	0.58	1.00	0.61	2.53	0,81
SEM	0.14	1.00	0.83	0.33	0.66	1.67	0.88	0.47	0.33	0.58	0.35	1.46	0.47
t-value		−0.6	−1.4	−2.3	−0.5	−0.8	1.8	−1.0	0.0	2.1	1.7	−0.1	2.7
F-ratio variances		13.5	9.3	1.5	6.0	37.4	10.5	3.0	1.5	4.5	1.7	28.8	2.9
GLOBAL SCORE													
Mean	5.9	6.2	4.9	6.3	5.7	4.7	7.3	6.5	6.7	5.1	6.0	5.8	6,1
Dev st.	2.30	4.60	2.20	2.89	1.13	4.56	2.52	3.91	2.08	0.23	2.97	4.10	3,78
SEM	0.66	2.66	1.27	1.67	0.65	2.63	1.45	2.26	1.20	0.13	1.71	2.37	2.18
t-value		0.10	−0.59	0.18	−0.17	−0.42	0.70	0.22	0.40	−0.61	0.01	−0.07	
F-ratio variances		4.01	1.09	1.58	4.16	3.93	1.20	2.89	1.22	99.16	1.67	3.18	

***P-level < 0.05.

[a] Standard error of the mean (SEM).

[b] Taster's code.

than the *supraspinatus* muscle, attributing scores significantly higher to the first cut except for appearance, which was evaluated similarly for both meat samples.

3.1.3. QDA and sensory profile of meat samples submitted to different tenderising technologies

The QDA was conducted on untreated and treated *semitendinosus* muscle samples tested after 2 (T_2), 24 (T_{24}) and 48 (T_{48}) h of storage at 3 °C. All results are shown in Table 3. Regarding the appearance attribute, judges gave a significantly higher score to the untreated meat samples for the whole storage time, which remained almost similar (P > 0.05). The evaluation of raw beef appearance

Table 2

Sensory descriptive tests of the *psoas major* **muscle** carried out during the training step: results of the Student t-test applied to the mean values assigned by each taster compared with the mean values of the whole panel.

	Panel	0002[a]	0004	0007	0008	0009	0010	0017	0011	0001	0016	0013	0015
APPEARANCE													
Mean	4.8	4.3	5.1	4.3	4.7	1.9	6.1	3.4	5	6.7	5.4	4.2	6.8
Dev st.	1.01	0.55	2.6	1.53	2.52	2.73	2.72	0.55	1.95	1.57	1.48	1.39	0.76
SEM[a]	0.29	0.32	1.50	0.88	1.45	1.58	1.57	0.32	1.13	0.91	0.85	0.80	0.44
t-value		−0.8	0.2	−0.5	−0.1	−1.8	0.8	−2.1	0.2	1.7	0.5	−0.6	2.7
F-ratio variances		3.37	6.61	2.28	6.19	7.29	7.24	3.37	3.72	2.41	2.15	1.88	1.75
COLOUR													
Mean	6.2	6.4	6.7	6.7	6	6.9	7	3	6.3	6.2	6	6.5	6.9
Dev st.	0.47	0.55	2.03	0.58	1.73	2.76	2	0.1	1.15	2.08	1	2.04	0.79
SEM													
t-value		0.3	0.3	0.8	−0.2	0.5	0.6	**−7.5**[b]	0.2	0	−0.3	0.2	1.1
F-ratio variances		1.79	7.57	1.63	5.53	14.03	7.38	**54.22**[b]	2.46	7.97	1.84	7.7	1.16
ODOUR													
Mean	5.4	5	5	4.3	5.3	4.4	7.1	5.6	5	7.2	6	3.9	6.6
Dev st.	0.28	0	1.15	0.61	1.47	4.09	2	1	1	1.93	1	0.98	0.71
SEM	0.08	0.00	0.66	0.35	0.85	2.36	1.15	0.58	0.58	1.11	0.58	0.57	0.41
t-value		**−2.84**[b]	−0.61	**−2.99**[b]	−0.18	−0.46	1.38	0.3	−0.76	1.55	0.91	−2.6	2.69
F-ratio variances		**0.00**[a]	17.41	**4.87**[b]	28.55	219.79	52.81	13.2	13.16	49	13.16	12.8	6.62
OFF FLAVOUR													
Mean	0.1	0	0	0	0	0	0	0.3	0	0	0	0.5	0
Dev st.	0.12	0.06	0	0	0	0	0	0.52	0	0	0	0.92	0
SEM	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.30	0.00	0.00	0.00	0.53	0.00
t-value		−0.51	−1.06	−1.06	−1.06	−1.06	−1.06	0.74	−1.06	−1.1	−1.06	0.86	−1.06
F-ratio variances		4.17	0	0	0	0	0	19.41	0	0	0	61.3	0
TENDERNESS													
Mean	6.3	6.6	6.4	6.3	5.3	4.7	7	6.5	7	5.7	6.3	6.7	7.2
Dev st.	0.25	0.58	1.18	0.58	2.56	4.51	1.95	0.72	1.76	1.15	1.46	1.65	0.76
SEM	0.07	0.33	0.68	0.33	1.48	2.60	1.13	0.42	1.02	0.66	0.84	0.95	0.44
t-value		0.74	0.1	0.1	−0.67	−0.63	0.59	0.38	0.72	−0.9	0.04	0.42	1.87
F-ratio variances		5.25	22.1	5.25	103.47	320.23	59.9	8.24	48.87	21	33.76	43	9.19
CHEWINESS													
Mean	6.3	6.2	6.4	6.7	5.6	4.7	6.9	6.4	6	6.7	6.3	6.4	7.2
Dev st.	0.21	0.59	1.18	0.58	2.12	4.51	1.85	0.75	1	1.55	1.47	1.71	0.76
SEM	0.06	0.34	0.68	0.33	1.22	2.60	1.07	0.43	0.58	0.89	0.85	0.99	0.44
t-value		−0.16	0.11	1.06	−0.53	−0.62	0.57	0.31	−0.49	0.49	0.01	0.11	1.91
F-ratio variances		7.76	31.72	7.54	101.8	459.65	77.54	12.73	22.61	54.6	49.05	65.8	13.19
JUICINESS													
Mean	6	6.2	6.4	6.7	5.6	4.7	6.9	6.4	6	6.7	6.3	6.4	7.2
Dev st.	0.15	0.59	1.18	0.58	2.12	4.51	1.85	0.75	1	1.55	1.47	1.71	0.76
SEM	0.04	0.34	0.68	0.33	1.22	2.60	1.07	0.43	0.58	0.89	0.85	0.99	0.44
t-value		0.61	0.5	1.88	−0.31	−0.52	0.82	0.94	−0.03	0.79	0.33	0.38	2.55
F-ratio variances		14.44	59.03	14.02	189.43	855.31	144.28	23.7	42.06	102	91.28	122	24.54
TASTE													
Mean	5.7	6.3	6	5.3	6	3.3	6.1	6.3	6.x0	5.97	6	5.5	6.5
Dev st.	0.19	1	1	1.15	1.73	2.89	1.06	1.36	1	1.67	1	1.8	0.56
SEM	0.05	0.58	0.58	0.66	1.00	1.67	0.61	0.79	0.58	0.96	0.58	1.04	0.32
t-value		0.47	0.42	−0.62	0.24	−1.45	0.61	0.65	0.42	0.22	0.42	−0.2	2.19
F-ratio variances		27.55	27.46	36.62	82.39	228.86	30.85	50.62	27.46	77	27.46	89.3	8.51
GLOBAL SCORE													
Mean	7.1	8.6	7.2	8.7	5	5	8.2	9	7.3	5.9	7.2	6.7	7
Dev st.	1.33	2.37	2.57	2.31	0.06	5	2.78	1.73	2.52	1.5	2.55	2.86	5.2
SEM	0.38	1.37	1.48	1.33	0.03	2.89	1.61	1.00	1.45	0.87	1.47	1.65	3.00
t-value		0.95	0.01	0.99	−2.76	−0.72	0.59	1.47	0.11	−1.1	0.03	−0.3	−0.05
F-ratio variances		3.18	3.73	3.02	529	14.18	4.38	1.7	3.59	1.28	3.7	4.63	15.31

***P-level < 0.05.
[a] Standard error of the mean (SEM).
[b] Taster's code.

depends on colour and texture changes. A significant effect was found for storage time on the colour score for untreated and treated samples. Moreover, untreated meat obtained a higher colour score than the treated sample after 2 and 24 h of storage. Nevertheless, the colour score increased after 48 h of storage in both samples, especially for those submitted to the innovative technological treatment of tenderisation (Table 3). The development of a typical cherry-red colour in meat depends on oxygen availability, oxygen diffusion into the beef and oxygen consumption rate. Under-vacuum packaging, meat oxygen decreases as vacuum packaging time increases, causing a worsening of the red colour. In our research, meat was underpacked in a plastic bag, and it is possible to speculate that the decrease in oxygen amount after 24 h of the colour of the meat was less vivid. Liu et al. [16] reported that packaging materials
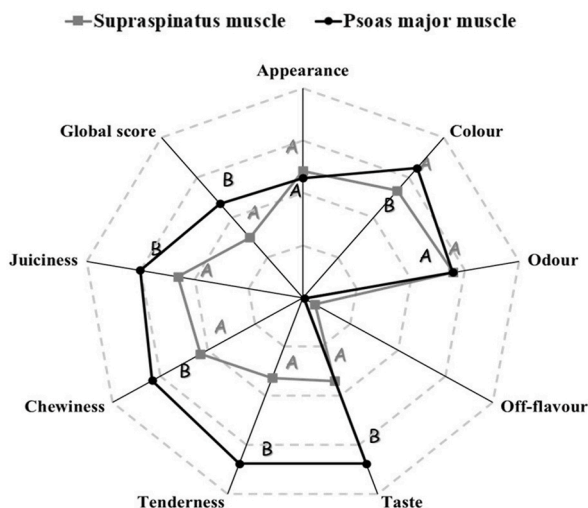
Fig. 5. Sensory profiles of the meat samples (Supraspinatus and Psoas major muscles) determined by the tasters during the six sessions of the training stage.

Table 3
QDA results of untreated and treated *Semitendinosus* muscle samples evaluated after 2 (T_2), 24 (T_{24}) and 48 (T_{48}) hours of storage at 3 °C.

	Storage time (h)	Samples		•MS_{error}
		Untreated	Treated	
Appearance	2	6.7 ± 1.01a[a]	5.5 ± 0.82b[a]	0.64
	24	6.8 ± 0.79a[b]	5.6 ± 0.47b	
	48	6.9 ± 0.90a[b]	5.7 ± 0.71b	
Colour	2	6.0 ± 0.56a	5.2 ± 0.55b	0.28
	24	5.8 ± 0.59c	5.1 ± 0.59d	
	48	6.0 ± 0.36e	6.2 ± 0.47f	
Odour	2	6.2 ± 0.79a	6.0 ± 0.93a	0.84
	24	6.5 ± 0.92a	6.2 ± 0.88a	
	48	6.6 ± 0.99a	6.1 ± 0.96a	
Off-flavour	2	0.4 ± 0.84a	0.1 ± 0.27a	0.18
	24	0.1 ± 0.05a	0.2 ± 0.45a	
	48	0.0 ± 0.08a	0.1 ± 0.27a	
Tenderness	2	5.5 ± 0.56a	7.2 ± 0.79b	0.65
	24	5.3 ± 1.02a	7.2 ± 0.73b	
	48	5.7 ± 0.79a	7.6 ± 0.88b	
Chewiness	2	4.9 ± 0.91a	6.9 ± 0.86b	0.77
	24	4.8 ± 0.99a	6.8 ± 0.80b	
	48	5.3 ± 0.72a	7.5 ± 0.94b	
Juiciness	2	5.4 ± 0.91a	5.0 ± 0.99a	0.62
	24	5.6 ± 0.69b	5.6 ± 0.57b	
	48	5.9 ± 0.82c	5.9 ± 0.66c	
Taste	2	5.7 ± 1.03a	6.0 ± 0.95a	0.73
	24	5.2 ± 0.75a	5.6 ± 0.59a	
	48	5.8 ± 0.91a	5.5 ± 0.83a	
Global score	2	5.4 ± 0.81a	6.5 ± 0.97b	0.66
	24	5.6 ± 0.74a	6.5 ± 0.50b	
	48	5.9 ± 0.82a	6.7 ± 0.95b	

***The interactions between treatment and storage were not statistically significant (P-level >0.05); therefore, they are not shown.
•Mean Squared Error (MS_{error}).
  [a] The means with standard deviation (SD) in the row followed by different letters significantly differ from each other (P-level <0.05, i.e., treatment effect).
  [b] The means with standard deviation (SD) in the column followed by different letters significantly differ from each other (P-level <0.05, i.e., storage effect).

can be considered a medium barrier if oxygen permeability values range between 5 and 200 cm^3/m^2·at 0.1 MPa·for 24 h. Consequently, the medium barrier to oxygen of the flexible film (<50 cm^3/m^2·at 0.1 MPa for·24 h) used to package samples favoured, after 48 h of storage, a gradual oxygen permeation inside the packaging.

Given that the appearance attribute considers colour and texture, data regarding colour demonstrate that the penalisation of the

appearance of the treated meat cannot be attributed to meat colour changes but, probably, to changes in texture linked to the structure of the fibres of the muscle bundles subjected to the combined treatment with ultra-sound and enzyme. Untreated and treated meat showed no statistically significant difference during storage time for odour, off-flavour and taste attribute scores (P > 0.05). No statistical difference was perceived by judges for the juiciness of untreated and treated meat. A statistically significant difference (P < 0.05) was observed for juiciness at different storage times. In particular, the perception of juiciness increased during storage time for untreated and treated meat. Treated meat obtained a higher score than the untreated samples for tenderness and chewiness attributes, while no significant differences were found during storage (Table 3). Consumers' satisfaction, primarily associated with tenderness, chewiness and juiciness, influences their intention to repurchase. Tenderness is considered the most crucial palatability trait [17].
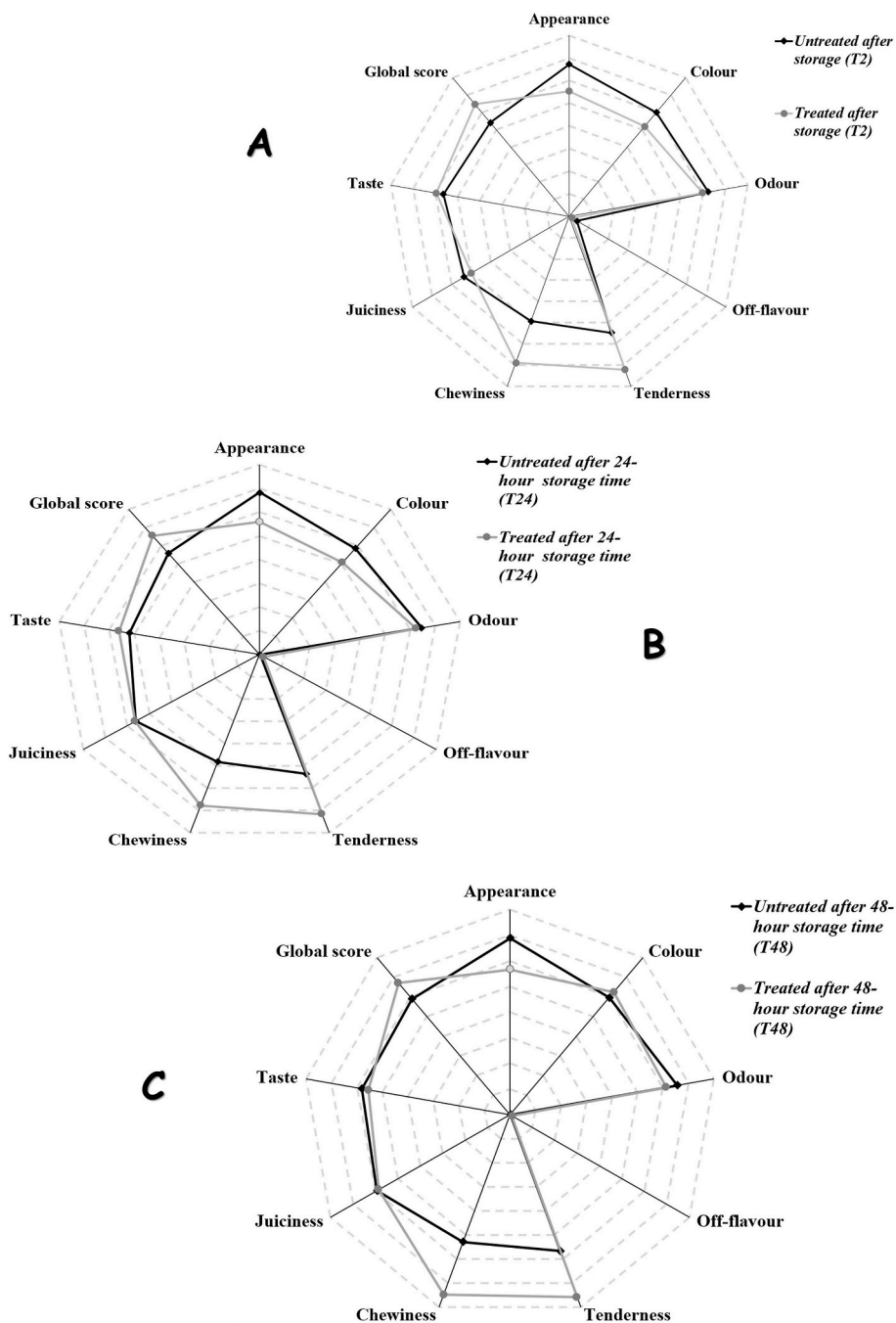
Fig. 6. Sensory profile of untreated and treated meat before storage **(A)** and after 24 **(B)** and 48 h **(C)** of storage at 3 °C elaborated through QDA results.

Many researchers have reported that the primary source of consumer complaints and the most common cause of failure to repurchase is tenderness variation, particularly the presence of toughness [18]. Consumers are willing to pay a premium for tender meats [19]. The tenderness is defined as the opposite force required to bite through the sample with the molars and can be related to those of connective tissue, myofibrils and sarcoplasmic proteins [20]. Choe et al. [21] suggested that the myofibrillar component contributes to meat tenderness more than the connective tissue. The sensory results obtained in this study highlight that the tenderising process proposed by Marino et al. [10] effectively accelerates the tenderisation of meat compared to the conventional process. These authors affirmed that ultra-sound applied before papain treatment could modulate the tenderisation rate, reaching the same tender improvement obtained by the papain treatment at the end of storage time but with a slower hydrolysing degree. This behaviour could be a determinant in preserving the meat structure regarding chewiness and myofibrillar protein degradation. In addition, the effects of this treatment are verifiable not only through a proteomic approach but also through sensory analysis. The judges attributed the highest global score values to treated meat that did not significantly change during storage time (Table 3). It is possible to speculate that these results were strongly conditioned by the high scores attributed by the judges to the tenderness and chewiness of the treated meat. Contrary to what was expected, the low score assigned to appearance did not negatively affect the global sensory evaluation of the treated sample meat. However, it is necessary to consider that appearance was evaluated on raw meat; if the assessment was made only on visual characteristics, it is possible to suppose that the global score of treated samples may be lower than that of untreated meat. Visual appearance is the consumer's primary and crucial characteristic for evaluating meat quality [22]. The purchase decision can be negatively influenced by any condition affecting the visual appearance of raw meat, causing an economic loss. Kuttappan et al. [23] highlighted an annual revenue loss of about $1 billion due to beef surface discolouration and the consequent need to apply a 15 % discount to sell such products. Therefore, it is essential to investigate the causes that led judges to evaluate the appearance of treated meat as worse than the untreated samples.

To better represent the QDA results, the obtained data were used to develop the sensory profiles of untreated and treated meat after 2, 24 and 48 h of storage (Fig. 6). Each sensory attribute was represented by an axis originating from the centre of the figure and corresponding to the zero point of the scale. The intensity of each characteristic increases in the direction of the edge of the graph. The mean score of each attribute is reported on the corresponding axis, and the sample sensory profile was drawn by connecting these points [24]. In the QDA profiles, the storage time did not exert significant effects on the sensory profile of untreated and treated samples except for the juiciness descriptor because the shape of the profiles is the same for each storage time (T_2, T_{24} and T_{48}).

Table 4
Results of physical and mechanical analyses of untreated and treated meat samples stored at 3 °C for 48 h.

	Storage time (h)	Samples		•MS_{error}
		Untreated	Treated	
L[a]	2	41.3 ± 0.28a[a]	44.1 ± 0.56b[a]	0.56
	24	44.3 ± 0.35c[b]	44.5 ± 1.53d	
	48	45.9 ± 0.71e[b]	46.7 ± 0.08f	
Hue angle	0	52.1 ± 0.56a	54.9 ± 1.24a	1.56
	24	51.5 ± 0.92b	50.3 ± 0.16b	
	48	46.5 ± 0.42c	46.2 ± 1.18c	
Chroma	2	14.6 ± 0.70a	15.7 ± 0.86a	0.74
	24	16.4 ± 0.75b	16.3 ± 1.13b	
	48	23.7 ± 0.64c	23.2 ± 0.81c	
WB Shear Force (N)	2	55.1 ± 2.36a	50.1 ± 1.63b	4.31
	24	41.5 ± 1.59c	36.7 ± 1.93d	
	48	34.9 ± 1.42e	25.5 ± 1.88f	
Hardness (N)	2	121.7 ± 11.62a	115.3 ± 10.24b	174.8
	24	109.7 ± 2.79c	84.2 ± 10.66d	
	48	96.8 ± 18.72e	54.4 ± 5.0f	
Cohesiveness	2	0.4 ± 0.02a	0.3 ± 0.05b	<0.01
	24	0.3 ± 0.01c	0.2 ± 0.01d	
	48	0.2 ± 0.02e	0.1 ± 0.01f	
Gumminess (N)	2	68.8 ± 3.34a	29.7 ± 1.42b	22.41
	24	36.6 ± 1.46c	15.0 ± 1.04d	
	48	32.3 ± 3.21e	3.2 ± 0.02f	
Chewiness (N[a]mm)	2	54.5 ± 4.64a	25.3 ± 2.37b	24.41
	24	32.9 ± 4.92c	13.8 ± 0.46d	
	48	25.2 ± 2.20e	5.3 ± 0.72f	
Springiness (mm)	2	8.0 ± 0.03a	7.9 ± 0.07b	0.98
	24	7.9 ± 0.02c	7.8 ± 0.01d	
	48	5.6 ± 0.15e	1.8 ± 0.08f	

***The interactions between treatment and storage were not statistically significant (P-level >0.05); therefore, they are not shown.

•Mean Squared Error (MS_{error}).

[a] The means with standard deviation (SD) in the row followed by different letters significantly differ from each other (P-level <0.05, i.e., treatment effect).

[b] The means with standard deviation (SD) in the column followed by different letters significantly differ from each other (P-level <0.05, i.e., storage effect).

Moreover, the sensory profiles highlight that tenderness and chewiness scores were higher for the treated meat. Moreover, the appearance of the treated sample obtained a lower score than that of the untreated one at each storage time (Fig. 6).

3.2. Relationship between sensory and instrumental data referred to meat samples submitted to different tenderising technologies

Results of physical analyses on chromatic characteristics show that luminosity (L*) increased during storage time for untreated and treated meat samples. Moreover, the L* values of meat submitted to ultra-sound radiation and enzyme injection were greater than those of the untreated samples (Table 4). The tenderising treatment did not affect the hue angle and chroma values for both samples, while a significant decrease in hue angle and an increase in chroma values were found during storage time (Table 4). These results highlight that the colour of meat samples improved as a consequence of oxygenation and transformation of myoglobin into oxy-myoglobin (blooming effect), favouring the increase of the red index by imparting a cherry-red colour to the meat [25]. The increase in L* and chroma values involved the improvement of luminosity and the increase in the pureness of red, thus causing a decrease in hue angle values.

The higher L* values of treated samples could be due to changes in myofibrillar structure caused by ultra-sound radiation. Kang et al. [26] observed the effects of ultra-sonic power on the meat micro-structure. The authors highlighted that the myofibrils were ruptured along with the z-lines, and the spaces between myofibrils were larger because of the ultra-sound treatment. Barekat & Soltanizadeh [27] made the same observations and considerations when conducting a histological analysis on untreated beef samples submitted to ultra-sound radiation and a combination of ultra-sound treatment with papain solution. The enlargement of the myofibril structure might increase the reflectance of the light beam during colourimetric determination.

The sensory and instrumental approaches data were also statistically assessed to determine a possible Pearson's correlation. Results revealed no significant correlation (P > 0.05) among sensory descriptors (appearance and colour) and chromatic indexes (L*, hue angle and chroma). Sensory descriptors such as colour and appearance depend on many factors influencing the judge's perception and evaluation. Moreover, these factors must be determined through different analytical indexes and techniques (meat structure determined by image analysis, colourimetric parameters by colourimeter, and so on). Furthermore, the under-vacuum packaging of meat represents a further source of variability because of changes that may occur in the muscle myofibrillar structure and the kinetics of oxygenation, making it extremely hard to find a linear correlation among sensory and instrumental evaluations. The results of the sensory and instrumental evaluation of chromatic characteristics did not explain the lower appearance score given to the treated samples by the trained panel. Consequently, the image analysis was conducted on the meat sections of untreated samples, and the meat was submitted to papain injection, ultra-sound radiation or a combination of enzyme and ultra-sound treatments. The effects of single and interactive treatments were evaluated to better understand what technological treatments caused the worsening of treated meat appearance. Fig. 7 shows the more compact structure of untreated samples than those submitted to each treatment. The observation of the cross-section of samples submitted to injection of the papain solution highlighted a collapsed structure, mainly in the peripheral
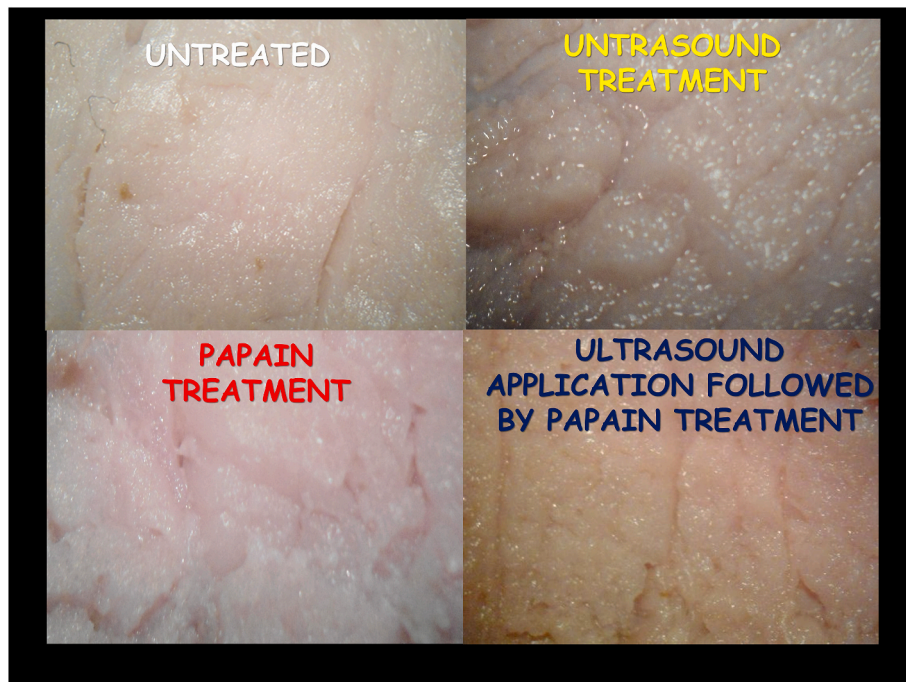

Fig. 7. Images of *semitendinosus* muscle (acquired by a stereo microscope equipped with a digital camera) submitted on different technological treatments.

area of the piece. The meat, submitted to ultra-sound radiation, showed a particular enlargement of the meat structure due to the spacing of the fibres. It is reasonable to suppose that the low score assigned by panellists to the treated sample appearance was due to the collapse of the structure caused by extremely intense technological treatments. For this reason, fine-tuning the tenderisation process is advisable, and it can be achieved by decreasing the amount of papain and the time and intensity of the ultra-sound treatment. It is also advisable to perform ranking tests to evaluate appearance and tenderness to choose the optimal operating conditions before proceeding to the industrialisation stage of this new technology.

The results of WBSF and TPA parameters highlight a progressive and quicker tenderising of the treated meat compared to the untreated one. WBS, hardness, cohesiveness, gumminess, chewiness and springiness of the treated meat were significantly lower than those of the untreated samples during storage (Table 4) and agreed with those of Marino et al. [10]. These authors found that ultra-sound applied before the enzyme injection favoured the increase of spaces between myofibrils with significant penetration of the papain solution inside the meat, thus improving its tenderness during storage.

The linear regression results highlight a significant negative correlation between WBS and juiciness ($R = -0.82$ and $P = 0.04$). Cohesiveness was negatively correlated with tenderness ($R = -0.81$ and $P < 0.04$), sensory chewiness ($R = -0.85$ and $P = 0.03$) and global score ($R = -0.88$ and $P = 0.02$). In addition, the global score was negatively correlated with gumminess ($R = -0.88$ and $P = 0.02$) and chewiness ($R = -0.85$ and $P = 0.03$). These results mean that roasted samples with low values of WBS, cohesiveness, gumminess and chewiness are perceived to have higher juiciness and tenderness and lower sensory chewiness. These data confirm that the treated samples are more tender and appreciated by panellists than the untreated ones, highlighting a better correlation between instrumental and sensory analysis. Nevertheless, the QDA analysis showed a meaningful tool to obtain information that is difficult to obtain by instrumental analysis. In fact, the critical aspect relating to the appearance of raw meat treated through innovative technology was detected only by sensory analysis.

4. Conclusion

The results confirm the beginning hypothesis, highlighting the importance of a correct QDA analysis application, which can provide information that is challenging to obtain through instrumental analyses. It can then be considered an indispensable and powerful tool for optimising new technology, such as tenderising meat by combining ultra-sound and papain injection. The instrumental analyses, in fact, did not notice the problem of the worsening of the appearance of raw meat in treated samples. The lack of this information could compromise the results of this innovation because appearance is a determinant factor in consumers' choice of meat. In addition, this information could permit modulating the technological parameters of the innovative process before proceeding with the industrial scale-up.

A possible prosecution of this study could involve the individuation of the optimal values of technological parameters used to accelerate the tenderness process by combining ultra-sound radiation and papain injection only through simple sensory analyses such as ranking tests as a function of the appearance of raw meat and tenderness on cooking meat that resulted in the characteristics that profoundly influenced the meat quality perceived by tasters.

Ethics statements

In Italy, sensory analysis tests conducted as part of scientific research do not have to receive mandatory approval before being carried out by an ethics committee if the material served to the tasters complies with all legal obligations relating to hygienic safety and health during the preparation and marketing phase. The same obligations are extended to all ingredients or experimental treatments the products tasted and underwent during the experimental phase. The research's meat objects are processed following conventional protocol productions, respecting all hygiene and safety procedures so as not to risk users' health.

The sensory study was performed using human volunteers who had previously been asked to sign an informed consent form. An appropriate protocol for protecting the rights and privacy of all participants was utilised (see supplementary contents named Annex II).

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Data availability statement

The data about this study have not been deposited in a publicly accessible repository, given that all relevant data are thoroughly detailed in the article, supplementary materials, or appropriately cited in the manuscript.

CRediT authorship contribution statement

Teresa De Pilli: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ofelia Alessandrino:** Data curation. **Antonietta Baiano:** Writing – review & editing, Writing – original draft, Visualization, Validation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32618.

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