TITLE

Near Infrared Reflectance Spectroscopy to analyze texture related characteristics of *sous vide* pork loin.

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ABBREVIATIONS

LT–LT: lower temperatures during longer times; TPA: Texture Profile Analysis; QDA: Quantitative Descriptive Analysis; NIRs: Near Infrared Reflectance spectroscopy (NIRs); WBSF: Warner Bratzler Shear Force; PC: principal components; PCA: principal component analysis; R: correlation coefficient; MASE: Mean Absolute Scaled Error**.**

ABSTRACT

This study aims to evaluate the ability of the Near Infrared Reflectance spectroscopy (NIRs) technique to analyze texture-related characteristics of sous-vide pork loins at different times of cooking. For that, pork loins were sous-vide at 70 °C for 1, 2, 4, 6 and 8h. Cooked samples were analyzed by means of NIRs, instrumental (cooking loss, pH, moisture, hydrolyzed collagen and texture profile analysis) and sensory analysis. Classification and predictive techniques of data mining were applied on the obtained data. Sous-vide loins were correctly classified as a function of time of cooking and their texture-related characteristics were predicted accurately, achieving correlation coefficients (R) higher than 0.5 and Mean Absolute Scaled Errors lower than 1 for most parameters. Thus, it is demonstrated the capability of NIRs to analyze most texture-related parameters of warm loin samples, and it may be recommended as a rapid and automatic techniques to stablish optimal cooking conditions of food.

KEYWORDS

Near Infrared Reflectance spectroscopy; texture-related characteristics; loin; sous-vide.

1. INTRODUCTION

Meats are usually cooked by pan-frying, stewing, roasting and grilling. These cooking methods apply high temperature (>100 °C) and short times (from minutes to 1–2 h). However, the current trend is cooking meat at lower temperatures during longer times (LT–LT), in order to improve the nutritional and sensory quality of cooked food. Catering services, food processing and chefs have been adopting this type of cooking, such as sous vide and confit (Sanchez del Pulgar et al., 2012). Sous vide cooking is defined as the cooking of raw materials under controlled conditions of temperature and time, inside heat-stable vacuumized pouches or containers followed by rapid cooling (Baldwing, 2012). Temperatures around 70 °C and times even more than 24 h are used for sous vide cooking meat (Ruiz et al., 2013).

Food cooking lead to physicochemical changes (in colour, composition and structure) and reactions (chemical and enzymatic), which depends on the type and conditions (temperature and time) of cooking, and they could even detriment the quality of food in the case of not being appropriate (Roldán et al., 2013). Most notable modifications during cooking of meat are related to protein denaturation, fiber shrinkage and collagen solubility (Tornberg, 2005), which mainly influence on texture-related parameters. In fact, it has been reported that changes in myofibrillar proteins increase toughening, while the solubilization of collagen has a tenderizing effect (Nikmarnam et al., 2011).

Regarding sous vide cooking, Sanchez del Pulgar et al. (2012) have indicated that it enhances solubilization of collagen, which is an indicator of tender meat and depends on total collagen content, types of collagen present, its solubility and cross linking of collagen matrix (Kanatt et al., 2015). Sanchez del Pulgar et al. (2012) have also pointed out the effect of the collagen content on instrumental textural parameters of sous vide cooked meat samples, finding a different influence of sous vide cooking on texture parameters from the Texture Profile Analysis (TPA) between beef and pork cheeks.

The sous vide cooking influences positively on sensory attributes, mainly improving meat tenderness and juiciness in comparison to conventional cooking techniques (Armstrong & McIleveen, 2000). These two sensory attributes are worth noting in sous vide cooked meat, since they depend on the collagen in connective tissue (Savell & Cross, 1988) and are very influencing factors of eating quality (Maltin et al., 2003).

Thus, the evaluation of texture-related parameters of sous vide cooked meat would help to optimize cooking conditions. Instrumental methods of texture and sensory evaluation have been extensively applied in many pork studies, mainly as a control quality tool. Instrumental texture methods are based on mechanical tests for measuring the resistance of the food to forces greater than gravity. Most common instrumental texture methods are Warner Bratzler test and TPA (Cavitt et al. 2004). Sensory evaluation results on objective information about the consumers' preferences and the sensory attributes of a product (Aaslyng et al., 2007; Nam et al., 2009), with the Quantitative Descriptive Analysis (QDA) being one of the most used in meat products (Perez-Palacios et al., 2011; Roldán et al., 2014).

All these techniques are laborious and time-consuming and, in the case of sensory analysis, requires a trained panel. At this respect, the use of alternative methodologies to predict quality attributes of meat as a function of data obtained from rapid and automatic techniques is being claimed (Pérez-Palacios et al., 2014; González-Mohino et al., 2018). In this sense, the use of Near Infrared Reflectance spectroscopy (NIRs) has been proposed to predict a number of physico-chemical and sensory parameters of fresh meat and meat products, i.e. pH, instrumental colour, fat content, drip loss, tenderness and juiciness in dry-cured loin and hams (García-Rey et al., 2001; Zamora-Rojas et al., 2011); sensory characteristics related to the eating quality (juiciness, flavour, abnormal flavour and overall liking) of lamb meat samples (Andrés et al., 2007). However, the prediction of quality characteristics of cooked meat by means of NIRs has been less studied, as reported by González-Mohino et al. (2018), who determine most sensory attributes in oven-cooked pork loins. These authors carried out the NIRs analysis in minced and cool samples, which can be an inconvenience when the objective is evaluating cooked samples.

Taking into consideration all these aspects, the present work aims to evaluate the capability of NIRs to analyse most texture-related parameters of pork loins during the sous vide cooking at different times. The influence of temperature and sample stage (sliced or minced) on NIRs absorbance values was firstly analysed.

2. MATERIAL AND METHODS

2.1. Experimental design

This study was carried out with fresh pork loins, bought in a local Spanish supermarket (Mercadona, Spain). The loins were sous-vide cooked at 70 ^{o}C during 1, 2, 4, 6 and 8 h. Three pieces of pork loins (400 ± 20 g) were cooked in different days at each temperature–time combination. The temperature of pork loins during cooking was recorded using a thermometer probe (Testo 735-2, Lenzkirch, Germany). From each sous-vide cooked loin piece, a central portion of 1 cm thickness was longitudinally sliced and analysed by NIRs technique in quintuplicate 5 min after cooking at a temperature around 66 ± 2 ºC. Next, pH, instrumental texture and sensory analysis were carried out. After that, sample was minced for determining moisture and hydrolysed collagen.

In addition, a preliminary assay was performed to evaluate the influence of sample temperature and preparation on NIRs absorbance values. For that, NIRs measurements (in quintuplicate) were carried in warm samples (70± 2 ºC), and after cooling at moderate (38 ± 2 ºC) and cold temperatures (4 ± 2 ºC) at two stages, minced and sliced (1 cm wide). A domestic meat grinder was used for mincing the samples.

2.2. Cooking loss

Each pork loin piece was weighted before and after cooking to measure cooking losses during sous-vide cooking.

2.3. pH

The pH of the sous-vide cooked pork loins was determined with meat pH meter electrode model FC232D (HANNA Instruments S.L., Eibar, Spain). The pH-meter was calibrated with commercial buffer solutions (Crison, Barcelona, Spain) at pH 4.0 and 7.0 prior to use.

2.4. Moisture

Moisture was analysed following AOAC (2000) reference method 935.29.

2.5. Hydrolysed collagen

Percentage of hydrolysed collagen in cooked pork loins was measured by determining hydroxyproline (HP) content in raw meat, cooked meat and cooked out juice according to the methodology described by Naveena et al. (2011) with some modifications. Sample preparation for raw meat consisted of hydrolysing sample (2 g) with HCl 6N (40 mL) at 108 ºC during 18h. The hydrolysate was filtered, and the volume adjusted to 50 ml with distilled water. Then 25 ml of hydrolysate was taken, pH was adjusted to 7.0 using NaOH 40% and distilled water was added until a volume of 50 ml. In the case of cooked loins, minced samples (5 g) was homogenized with 50 ml distilled water at 4±1 °C in a blender for 2 min. The extract was then centrifuged (4000 rpm, 30 min). Finally, the supernatant was hydrolysed for 18 h at 108 °C in hot air oven. As for cooked out juice, sample (25 ml) was also hydrolysed in hot air oven for 18 h at 108 °C. Official method for measuring HP content was applied (AOAC, 2000, reference method 990.26), using one millilitre from each solution and a standard curve of HP (5-20 µg HP mL^{-1}). Absorbance was measured at 560 nm using UV–VIS spectrophotometer (Model UV-

1800, SHIMADZU, Japan). Percentage of hydrolysed collagen was calculated by multiplying 7.14 with percentage of HP solubilized, which was calculated as follows:

HP solubilized (%) = (g HP in cooked out juice + g HP in cooked meat) / (g HP in raw meat) × 100

Hydrolysed collagen (%) = 7.14 x HP solubilized (%)

2.6. Instrumental texture

Texture analysis was performed in a TA.XT plus Texture Analyser (Stable Micro Systems Ltd., Surrey, UK). For determination of the TPA, uniform portions of the sous-vide cooked samples were cut into 1 cm^3 cubes. Samples were axially compressed to 60% of their original height with a flat plunger 50 mm in diameter (P/50) at a crosshead speed of 2 mm/s through a 2-cycle sequence. The following texture parameters were measured from the force deformation curves (Bourne, 1978): Hardness (g) = maximum force required to compress the sample (peak force during the first compression cycle); adhesiveness (g x s) = work necessary to pull the compressing plunger away from the sample; springiness (dimensionless) = height that the sample recovers during the time that elapses between the end of the first compression and the start of the second; cohesiveness (dimensionless) = extent to which the sample could be deformed before rupture (A1/A2, A1 being the total energy required for the first compression and A2 the total energy required for the second compression); chewiness (g) = the work needed to chew a solid sample to a steady state of swallowing (hardness x cohesiveness x springiness). For Warner Bratzler Shear Force (WBSF) analyses, samples (1x10x1 cm^3 slices, thickness x length x width), were cut with a Warner-Bratzler blade (HDP/BS) perpendicularly to the muscle fibres, determining firmness (g) = maximum force to cut the ample and toughness (g x s) = work needed to cut the sample. In both analysis, determinations were repeated ten times per sample and were averaged.

2.7. Sensory analysis

Sous-vide cooked loin samples were subjected to a descriptive sensory evaluation, particularly quantitative-descriptive analysis of texture attributes. Fourteen trained panellists were used for this purpose. All of them were staff at the Meat and Meat Products Research Institute (IProCar) of the University of Extremadura. Samples were evaluated during five sessions, with three products tasted per session and evaluating each time loin in triplicate. After cooking, the sous-vide cooked samples were refrigerated for 24 h until sensory evaluation. Then, loins were sliced using a slicer meat machine TGI 300 OMS S.r.l. (TGI, Jerago con Orago, Italy) (slice samples of 2mm and around 5 g). Just before the evaluation, samples were heated for 10 s in a

600W microwave oven. Samples (one slice per plate) were served on glass plates with a mineral water and a piece of unsalted cracker to follow the rinsing protocol between samples. Evaluations were developed in tasting rooms designed according to the UNE-EN-ISO 8589:2010 regulation. All sessions were conducted at room temperature (20–22 ^{o}C) in a sensory room equipped with white fluorescent light. The serving order of the samples was randomized according to the Williams Latin square design. FIZZ software 2.20 C version (Sensory Analysis and Computer TestManagement) (Biosystèmes, Couternon, France, 2002) was used for collecting the data.

Attributes used in this study were selected based on the previous experience of the authors in sensory evaluation of meat products (González-Mohino et al., 2018) and according to a tasting evaluation of the cooked samples in a previous pilot study. The following texture attributes were chosen: hardness (effort required to bite through sample and to convert it to a swallowable state), juiciness (impression of lubricated food during chewing), fibrousness (extent to which fibres are perceived during chewing) and chewiness (number of chews or time of chewing required to masticate the product). A 10 cm unstructured scale was used for attributes scoring, and verbal anchors were fixed as 'little' to 'very much' for all evaluated attributes. Acceptability of the samples was also scored under the same evaluation conditions.

2.8. Near-infrared spectroscopy analysis procedure

Immediately after cooking during different times, a slice of sous-vide cooked pork loin was analysed by NIRs (FOODSCAN lab, Foss, Hillerod, Denmark) using a wavelength range of 850–1048 nm and obtaining the spectra by absorbance. Each sample was analysed in quintuplicate. First, spectral data were imported from .nirs to .jcamp extension by using WinIsi III (Foss, Hillerod, Denmark), in order to allow extraction of numerical data from the near-infrared spectra. Then, the spectra were pre-processed by removing the noise by using Unscrambler v. 10.4.1 (Camo Software, Oslo, Norway) with the filter MSC. Thus, the outlier spectra were eliminated. Figure 1 shows original spectral graph before (A) and after (B) pre-processing. Finally, mean values for bandwidth each 10 nm were calculated from the near-infrared spectra.

2.9. Statistical analysis

Time of sous-vide cooking effect on physico-chemical and instrumental texture parameters was analysed by one-way variance analysis (ANOVA), applying the Tuckey's test when finding significant effect ($p \leq 0.05$). In the case of sensory data, the Kruskal–Wallis test followed by the

Mann–Whitney U test were applied when significant differences ($p \leq 0.05$) were found. Principal Components Analysis was applied on NIRs absorbance values from cooked loin samples differing on NIRs analysis conditions (temperature and preparation), and on data from texture-related parameters and NIRs analysis of pork loins sous-vide at different times. Parameters showing the highest values in each matrix component were selected as the most influencing ones. Pearson's correlation was also calculated between texture-related parameters and NIRs absorbances. The IBM SPSS v.22 (IBM Co., New York, USA) statistics software package was used to carry out these analyses.

Prediction of texture-related parameters of sous-vide pork loin by means of NIRs absorbance was carried out by means of predictive techniques (Multiple Linear Regression, MLR) of data mining, using the free software Waikato Environment for Knowledge Analysis (WEKA; http://www.cs.waikato.ac.nz/ml/weka). The M5 method was applied for attribute selection, and a ridge value of 1.0×10^{-4}. This method is based on removing the independent variable with the smallest standardized coefficient. Then, the independent variable is dropped if the result is improved. This was repeated until no improvement was observed in the estimation of the error (Grossman et al., 2010). The correlation coefficient R was used for evaluating the accuracy of fit of the prediction according to rules given by Colton (1974): R=0 0.25, 0.25–0.5, 0.5–0.75 and 0.75–1 indicate very low, low, acceptable and high correlations, respectively. The Mean Absolute Scaled Error (MASE) (Hyndman & Koehler, 2006) was calculated to validate the prediction results too. The MASE measures the difference between real and predicted values with independence of the scale data.

3. RESULTS AND DISCUSSION

3.1. Texture-related parameters of sous-vide pork loin as affected by cooking conditions

Table 1 shows pH, percentage of moisture and cooking loss in the sous-vide pork loins cooked during different times. pH values did not vary among samples. As expected, percentage of moisture significantly decreased as the time of sous-vide cooking increased. Time of sous-vide cooking also influenced on cooking loss that increased from 1 to 2 h and then are maintained until 8 h. Similarly, in LT-LT cooked meat from pork, lamb and beef (Sánchez del Pulgar et al., 2012; Roldán et al., 2013; Christensen et al., 2013), cooking loss increased and moisture decreased with longer heating times. Most of the water of muscles is retained by the myofibrillar proteins but, at temperatures above 60 ^{o}C, such proteins shrink notably and, consequently, causes significant water losses (Lepetit et al., 2000). However, this contraction seems to occur to a lesser extent in LT-LT meat samples. This has been related to temperature of the denaturation of the tropocollagen (needed for the contraction of the connective tissue) in these kind samples, which is 68 ºC. In fact, according Roldán et al. (2013), temperature of sous vide cooking of 70 ºC and above causes higher cooking losses than 60 ºC during longer time. These aspects may also explain the increase of cooking losses of the present work, being more notable at shorter times of sous-vide cooking at 70 ºC.

Percentage of hydrolysed collagen (Table 1) of sous vide pork loin showed significant differences due to time of cooking, gradually increasing from 1 to 4 h and then maintaining from 4 to 6 and to 8 h. This agrees with Christensen et al. (2013) who found that the solubility of collagen increased with increasing heating time (from 2 1/2 h to 19 1/2 h) at 58 °C in meat from young bulls. These findings are explained by the collagen denaturation, which takes place at around 53-63 ºC (Martens et al., 1982). It may initiates breaking the hydrogen bonds and loosing up the fibrillary structure, and it ends with the contraction of the collagen. At higher temperatures (60-70 ºC), collagen fibres contract and shorten until one-quarter of its resting length (Tornberg, 2005). It has also been described connective tissue contracts to a lesser degree in LT-LT treated meat samples. In pork cheeks, Sánchez del Pulgar et al. (2012) observed collagen fibers not completely denatured when cooking at 60 ºC-12h in comparison to samples cooked at higher temperature. This has been related to the action of muscle enzymes that disintegrate collagen structure before its contraction, which, consequently, will be less intense (Christensen et al., 2013). In these types of treatments, collagenase activity may hold for up to 6 h (Christensen et al., 2013), which could also explain results on hydrolysed collagen of the present work.

Results on instrumental texture analysis are shown in Table 1, finding the significant effect of time of sous vide cooking on most parameters. Regarding WBSF, firmness experimented a notable increased from 1 to 2h, and then it maintained; toughness also increased from 1 to 2 h, and maintained at 4, 6 and 8 h. As for the TPA, hardness increased from 1 to 2 h, maintained from 2 to 4 and 6 h and decreased to 8 h; the highest values of cohesiveness were found at short cooking times, following by 4 and 6 h of sous vide cooking and having the lowest cohesiveness values at 8 h; chewiness has shown a similar trend than hardness, increasing from 1 to 2 h, and progressively decreasing with the cooking time, especially at 8h. Previous works have reported the decrease of hardness, cohesiveness, chewiness and shear force as the heating time of sous vide cooking increased from 6 to 24 h (Roldán et al., 2013; Christensen et al., 2013). This is in concordance with the results of the present study that have shown the increase of values of most instrumental texture parameters from 1 to 2 h, and their decrease or maintenance at longer times (4-8h). Thus, considering the different influence between sous vide cooking for 1-2 h and for longer times, it is worth noting the importance of analyse the samples sous vide cooked at shorter times.

Meat tenderness is positively associated with connective tissue solubilization, sarcoplasmic proteins aggregation and collagenolytic activity, while denaturation of myofibrillar proteins and great water losses contribute to meat toughening (Baldwin, 2012). Roldán et al. (2013) have associated the decrease in hardness and shear force with the time of sous vide cooking to a greater collagen solubilization and no further increase of myofibrillar shrinkage at longer times. According to this, in the present study, changes in the percentage of hydrolysed collagen among samples sous vide cooked during 2, 4, 6 and 8h correlated negatively to firmness, toughness and hardness. However, 1h cooked samples do not follow this trend, and obtained the lowest values for these instrumental texture parameters and for hydrolysed collagen and cooking loss. Tornberg (2005) pointed out that the low stress needed to compress raw meat is due to the viscous flow in the fluid-filled channels in between fibres and fibre bundles. A similar behaviour may occur in the 1h sous vide cooked samples of this study, since they have experimented a slighter cooking loss than the samples cooked during longer times.

Sensory texture-related parameters of sous vide pork loin cooked at different times are exposed in Figure 2. Time of sous vide cooking have significantly influenced on all of them. Thus, sous vide cooked samples during 2 and 6 h obtained the lowest scores for hardness, chewiness and fibrous, while pork loins cooked during 4 and 8 h obtained the highest scores for these attributes and 1h samples showed intermediate values. Regarding to juiciness, 2h sous vide samples had the highest scores, followed by loins cooked during 1h, and those cooked at longer times (4, 6 and 8h) showing the lowest values for this texture sensory attribute. Besides the sensory analysis for studying texture-related attributes, a test for evaluating the effect of time of sous-vide cooking on the acceptability of pork loins was also carried out. The highest acceptability scores were found in pork loins sous vide cooked during short times (1 and 2 h). This is in concordance with data obtained from the quantitative-descriptive analysis (Figure 2), with the 2h sous vide loins obtaining high juiciness and low hardness, fibrousness and chewiness, which should be desired attributes in this kind of food (Schafheitle, 1990). Thus, according to our results, the application of shorter times (2h) for sous vide cooking pieces of pork loins of around 400 g should be indicated to achieve accurate acceptability and texture attributes. As our knowledge, there is not available information regarding the influence of sous-vide cooking conditions on meat samples. However, the study of Díaz et al. (2008) demonstrated the effectiveness of the sensory analysis for determining the shelf life of refrigerated sous vide pork-based dishes. Moreover, Naveena et al. (2016)

found improved sensory attributes in sous vide cooked chicken at 100 ^{o}C for 30, 60 and 120 min.

In addition, a PCA has been carried out with the texture-related parameters evaluated in sous vide cooked loins at different times (Figure 3). Two first principal components accounted by 68.77% of the total variance (34.00% for the PC1 and 24.77% for the PC2). Sous vide samples cooked at different times were separated in the plot, 1h and 2h cooked samples were in the left lower and upper quadrants, respectively, whereas those loins sous vide cooked at longer times were in the right quadrants, 4 and 8h sous vide loins in the lower and those cooked during 6h in the upper one. Most influencing texture-related parameters were: moisture, negatively loaded in PC1; cooking loss, hydrolysed collagen, toughness and adhesiveness, positively correlated with PC1; and hardness, fibrousness and chewiness that positively defined by PC2. Thus, loins sous vide at shorter times, specially 2h cooked samples, are mainly characterized by low values of cooking loss, hydrolysed collagen, toughness, adhesiveness, hardness, fibrousness and chewiness and high percentage of moisture, whereas longer times of sous vide cooking led to samples with high values for most texture-related parameters and low moisture. This is in concordance with above results and point out the significance of the texture-related parameters to categorize sous-vide pork loins as a function of cooking time.

3.2. Analysis of texture-related parameters of sous-vide cooked loins by means of NIRs absorbance

Once verified the influence of the time of sous vide on most texture-related parameters of loins, the capability of NIRs technology to analyse these quality characteristics were evaluated. In order to establish most convenience conditions for NIRs analysis for monitoring the quality characteristics of pork loin during cooking, the influence of sample temperature during the NIRs analysis and sample preparation was firstly studied in this work. Figure 4 shows PCA plots of NIRs absorbance values for sous-vide cooked pork loins as a function of temperature of analysis and sample preparation. In the case of the influence of the sample temperature (Figure 4.a), two first principal components accounted the 96.23 % of the total variance (81.69 % for the PC1 and 14.54 % for the PC2). Samples analysed at 70 and 37 ^{o}C were located at the upper quadrants (left and right), while those samples analysed at 4 ^{o}C were mainly found in the lower right quadrant. Regarding the influence of the sample preparation (Figure 4.b), 96.34 % of the total variance was accounted by PC1 (80.41 %) and PC2 (13.93 %), not having a clear separation between sliced and minced samples. These findings indicate that NIRs absorbance values of sous-vide cooked loins depends on sample temperature, with notable differences between chilled (4 ºC) and heated (37-70 ºC) samples. This agrees with Campos et al. (2018) who found variations in the absorption bands of the NIRs spectrum from dry-cured ham slices analysed at different temperature. However, the effect of sample preparations was not so notable, without differences between the use of sliced or minced samples. From a practical point of view, the use of sliced samples should be recommended since this way of sample preparation is more convenient and handier, and it also reproduces the usual mode of consumption this kind of food. In fresh pork, beef and lamb analysed by NIRs, several authors have detected better prediction results when using minced samples in comparison to the whole piece (Cozzolino et al., 2002; Prevolnik et al., 2005), but data about the effect of cooked sample preparation on NIRs analysis have not been reported until now.

Considering these findings, the use of sliced and warm-temperate samples seems to be appropriate to monitor quality characteristics of cooked samples by NIRs. Accordingly, samples of the present study were analysed in this way.

In order to locate most influencing wavelengths bands of the NIRs analysis on sous-vide loin samples, a PCA with data from NIRs spectra of sous-vide loins cooked at different times was conducted (Figure 5). PC1 and PC2 accounted by 58.28 and 22.29 % of the total variance. Three groups of bands were found, 880-890 and 960-980 nm, loaded on the positive axis of PC1, 1010-1030 nm, on the negative axis of PC1, and 910-920 nm, on the positive axis of PC2. Bands of 880-890 nm and 910-920 nm are within the third overtone and are related to C-H bonds. Bands of 960-980 nm and 1010-1030 nm are in the second overtone and can be ascribed to 0-H and N-H bonds, respectively. Prieto et al. (2017) have shown similar NIRs spectrum for fresh pork, beef and lamb, with four main bands at around 940-995, 1130-1240, 1380-1550 and 1870-1980 nm. Moreover, quite in agreement with data found in the present work, in the study of González-Mohino et al. (2018) with cooked pork loins, only one band has been found at 940-1040 nm. This PCA (Figure 5) also showed the score plot of sous vide cooked loins, finding a quite clear separation of samples as a function of time of sous-vide cooking. Samples cooked during 8h were in the upper quadrants, and those cooked for 4 and 6 h were located near to the central point, whereas sous-vide loins cooked for 1 and 2 h were in the low right and left quadrants, respectively. This result demonstrated the ability of NIRs to classify sous-vide loin samples as a function of the time of cooking.

After seeing the performance of sous-vide loins cooked during different times regarding the NIRs analysis, predictive technique of data mining was applied to obtain prediction equations of texture-related parameters as a function of NIRs absorbances. Table 2 shows the values of the correlation coefficients (R) and MASE for the prediction equations of each texture-related parameters of sous-vide cooked pork loins. Prediction equations of seven in fifteen texture-related parameters (moisture, cooking loss, hydrolysed collagen and sensory attributes (hardness, juiciness, fibrous and chewiness)) showed high correlation coefficients (R>0.75). Acceptable correlations (R=0.5-0.75) were found for pH and four instrumental texture parameters (firmness, hardness, adhesiveness and chewiness), while only the prediction equations of three characteristics (thoughness, springiness and cohesiveness) obtained low correlation (R=0.25-0.5). The low correlation obtained in these texture characteristics could be ascribed to the high homogeneity among the values of the different batches, being necessary a major numerical heterogeneity to achieve high correlation coefficients (Almeida de Macedo et al., 2013). Besides, most texture related parameters showed good MASE values (<1) according to Hyndman and Koehler (2006). Also, to validate the proposed prediction equations, real and predicted values of texture-related parameters were statistically compared (Table 2). No significant differences ($p > 0.05$) were found in all cases, which indicates an accurate adjust. These findings point out the accuracy of NIRs to predict texture-related parameters of cooked loin by analyzing warm samples. Previous studies have also shown the accuracy of NIRs to predict texture parameters of pork (Moteiro et al., 2015) and lamb meat (Andrés et al., 2007). However, these studies carried out the NIRs measurements on cold samples.

Giving a step forward in this analysis, the standardized coefficient Beta of the variables (wavelengths taken each 10 nm) of each prediction equations were calculated, trying to find out the importance of each independent variables in the prediction equations. Although Beta values were different among all prediction equations, it can be get some commonalities. Thus, in the prediction equations of pH, moisture, cooking losses, hydrolysed collagen and of most instrumental texture parameters, there are more wavelengths with high Beta values (0.5-0.8) between 850-960 than between 970-1040 nm. In the case of the prediction equations of sensory attributes, higher Beta values are found for the wavelengths between 950-1040 nm than for the former ones except for hardness, which showed the contrary behavior. Thus, it would not ease to select a range of wavelength bands for all sensory attributes.

Taking into consideration i) correlation coefficients and Beta values of the prediction equations as well as the influence of the different physico-chemical texture parameters and NIRs wavelength bands on discerning sous-vide loins regarding the time of cooking, the monitorization of sous-vide cooked loins should be based on prediction of cooking loss and/or hydrolyzed collagen by NIRs absorbance between 850-960 nm. Nevertheless, further studies on this subject should be done.

CONCLUSIONS

The use of different times of sous-vide cooking notably modifies most texture-related parameters of loins, with moisture, cooking loss, hydrolysed collagen, toughness and adhesiveness being the most influencing ones.

Temperature of NIRs analysis of sous-vide cooked loins influences on absorbance values, while the effect of sample presentation is not so marked.

The use of NIRs to analyse warm samples achieve to classify sous-vide loins as a function of time of cooking and predict most texture-related parameters of these samples with high accuracy.

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FIGURE CAPTIONS

Figure 1. Original spectral NIRs graph before (A) and after (B) pre-processing.

Figure 2. Results on sensory analysis of texture attributes of *sous-vide* pork loin cooked at different times.

Figure 3. Principal Component Analysis (PCA) of NIRs absorbance and with biplot for texture-related parameters for the two first principal components: effect *sous-vide* pork loin cooked at different times (1h (■), 2h (♦), 4h (▲), 6h (●) and 8 h (X)).

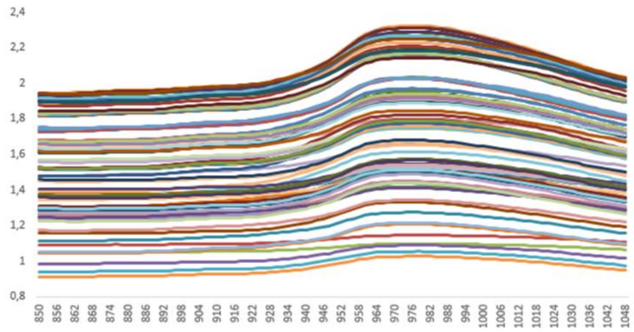
Figure 4. Principal Component Analysis (PCA) of NIRs absorbance with factor scores plot for the two first principal components: (a) effect of temperature of sample NIRS analysis (88 ºC (◊), 37 ºC (○), 4 ºC (●)) (b) effect sample preparation (sliced (■), minced (□)).

Figure 5. Principal Component Analysis (PCA) of NIRs absorbance and with biplot for wavelength bands for the two first principal components: effect *sous-vide* pork loin cooked at different times (1h (■), 2h (♦), 4h (▲), 6h (●) and 8 h (X)).

Figure 1

Figure 1.

A



B

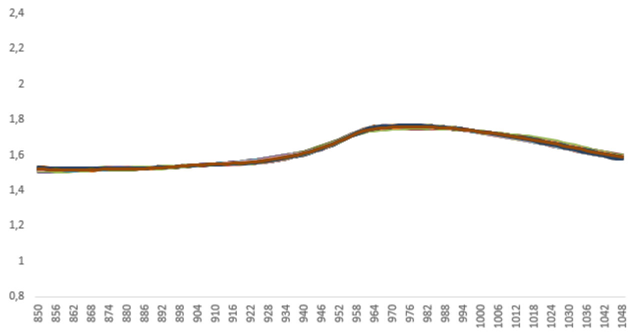
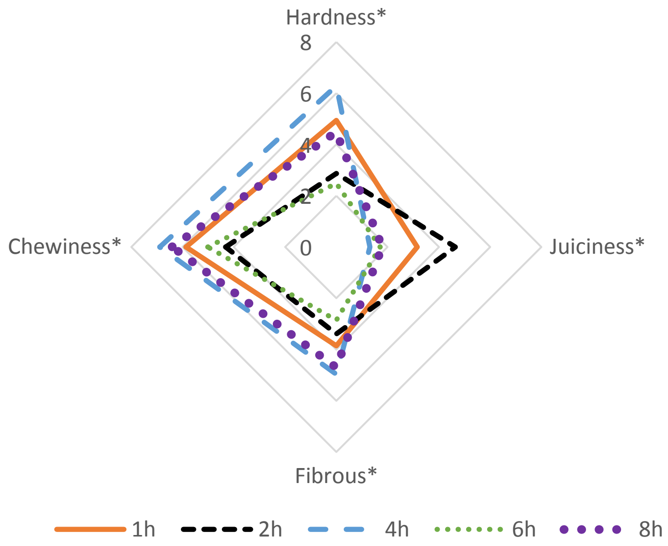


Figure 2

Figure 2.



*=statistical differences between samples (p<0.05)

Figure 3

Figure 3.

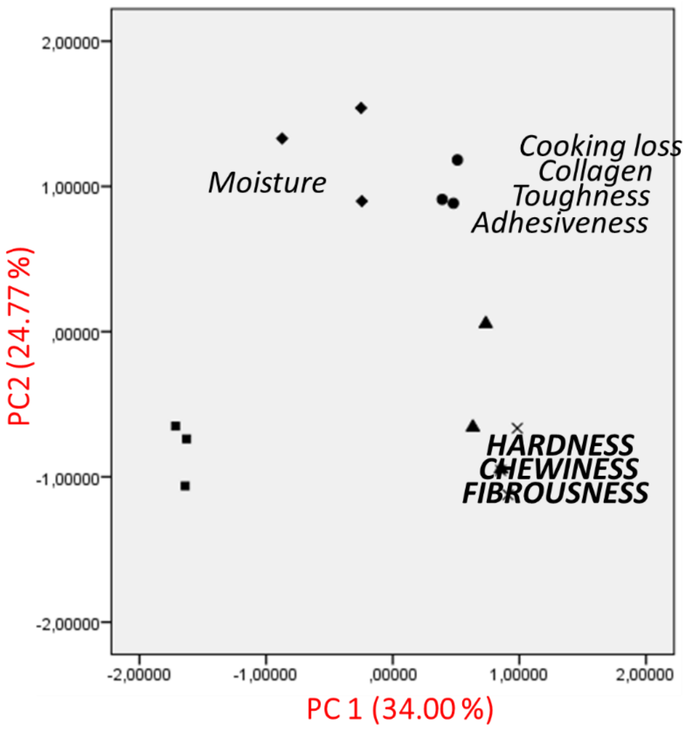
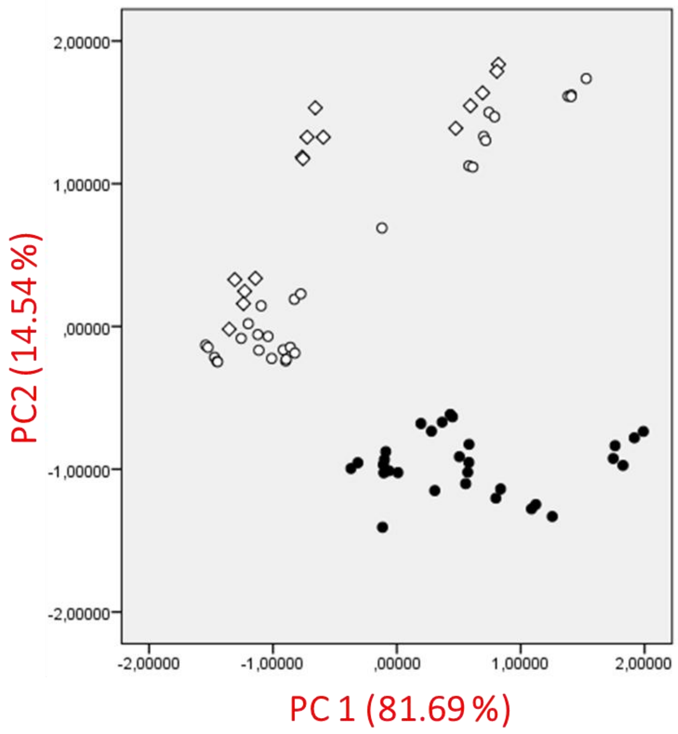


Figure 4

Figure 4.

a)



b)

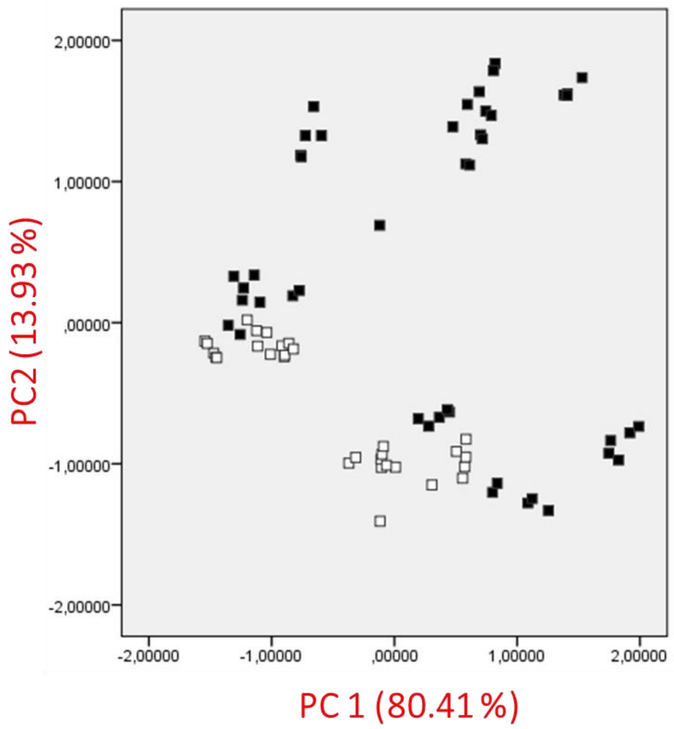


Figure 5

Figure 5.

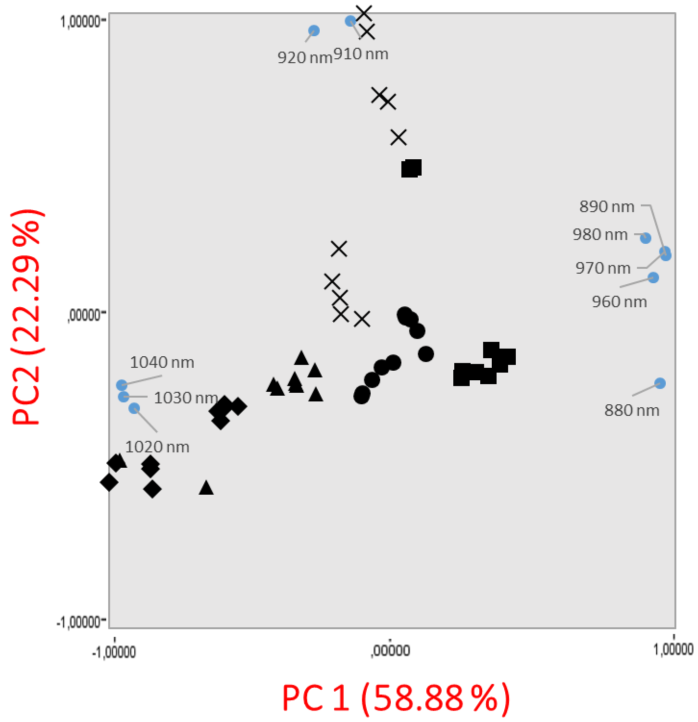


Table 1

Table 1. Values of pH, moisture, cooking loss and hydrolysed collagen and instrumental texture parameters of *sous-vide* pork loin cooked at different times*.

	1h	2h	4h	6h	8h	*p*
pH	5.64 ± 0.17	5.74 ± 0.04	5.75 ± 0.02	5.66 ± 0.01	5.73 ± 0.02	0.225
Moisture (%)	63.79 ± 2.06 a	62.57 ± 1.37 ab	60.64 ± 0.91 bc	59.86 ± 0.46 cd	57.98 ± 1.23 d	0.003
Cooking loss (%)	19.41 ± 2.78 b	30.61 ± 2.38 a	32.60 ± 1.26 a	34.82 ± 0.87 a	33.91 ± 1.51 a	<0.001
Hydrolysed collagen (%)	16.96 ± 0.18 c	23.88 ± 2.41 b	29.97 ± 2.81 a	33.37 ± 3.51 a	33.29 ± 1.19 a	<0.001
Warner Bratzler Shear Force						
Firmness (N)	35.84 ± 2.19 d	64.35 ± 3.94 a	59.19 ± 3.19 ab	50.48 ± 1.65 c	56.19 ± 1.04 bc	<0.001
Toughness (N x s)	311.27 ± 13.42 b	468.13 ± 20.40 a	461.00 ± 23.89 a	484.60 ± 5.32 a	479.28 ± 9.71 a	<0.001
Texture Profile Analysis						
Hardness (N)	43.44 ± 3.51 b	65.09 ± 5.55 a	67.37 ± 3.45 a	63.20 ± 7.96 a	46.73 ± 3.17 b	<0.001
Adhesiveness (N x s)	-0.04 ± 0.02 e	-0.01 ± 0.00 d	<0.01 c	<0.01 b	<0.01 a	<0.001
Springiness	0.46 ± 0.03	0.52 ± 0.04	0.46 ± 0.07	0.50 ± 0.08	0.44 ± 0.11	0.386
Cohesiveness	0.49 ± 0.03 a	0.50 ± 0.05 a	0.47 ± 0.03 ab	0.46 ± 0.03 ab	0.41 ± 0.05 b	0.023
Chewiness (N)	9.02 ± 1.31 bc	16.97 ± 5.70 a	14.51 ± 2.01 ab	11.51 ± 2.49 abc	6.98 ± 0.78 c	<0.001

*Values are expressed as mean ± standard deviation.

p values lower than 0.05 indicate a significant effect due to the time of cooking.

Different letters (a, b, c) in the same line indicate significant differences between samples.

Table 2

Table 2. Correlation coefficient (R) and Mean Absolute Scaled Error (MASE) of the prediction equations and p-value between real and predicted results of the texture-related parameters of *sous-vide* pork loins.

		R	MASE	*p*
Physico-chemical analysis	pH	0.50	0.064	0.872
	Moisture	0.83	0.054	0.828
	Cooking loss	0.97	0.459	0.906
	Hydrolysed collagen	0.96	0.189	0.868
Instrumental texture analysis	Firmness	0.74	0.672	0.904
	Toughness	0.47	0.371	0.921
	Hardness	0.67	0.572	0.943
	Adhesiveness	0.69	0.077	0.999
	Springiness	0.33	0.069	0.995
	Cohesiveness	0.26	0.068	0.992
	Chewiness	0.62	0.271	0.999
Sensory analysis	Hardness	0.76	0.480	0.911
	Juiciness	0.92	0.390	0.955
	Fibrous	0.80	0.160	0.965
	Chewiness	0.78	0.569	0.954

p values lower than 0.05 indicate significant differences between real and predicted values.

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6. Manuscript Identifying Number (if you know it)
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