Elsevier Editorial System(tm) for Food Research International

Manuscript Draft

Manuscript Number: FOODRES-D-17-02596R2

Title: Relating texture perception of cooked ham to the bolus evolution in the mouth

Article Type: SI: 5th ICFD

Keywords: Cooked ham; Texture; Bolus; Oral Processing

Corresponding Author: Dr. Amparo Tarrega, Ph.D.

Corresponding Author's Institution: IATA-CSIC

First Author: Arantxa Rizo

Order of Authors: Arantxa Rizo; Esmeralda Pena; Alma Delia Alarcon-Rojo; Susana Fiszman; Amparo Tarrega, Ph.D.

Manuscript Region of Origin: SPAIN

Abstract: The aim of the present study was to characterize the dynamics of the bolus formation that take place during mastication of commercial cooked ham samples. In addition, the relationships between these properties and texture perception were studied. Five commercial samples which presented different mechanical properties and moisture contents were studied. Ten participants were asked to chew the cooked ham samples normally and to expectorate the bolus after different chewing periods. Oral activity measurements (chewing time and number of chewing strokes), moisture content, saliva uptake and particle size distribution in the boluses were obtained. Seventeen participants evaluated the sensory perceptions generated over the sample consumption time, using the Temporal Check-All-That-Apply (TCATA) method. The results revealed that the duration of mastication and number of chewing cycles through to swallowing varied significantly among the cooked ham samples and were mainly related to instrumental texture parameters. The pattern of fragmentation under mastication also varied greatly between samples. Sensations of softness and hardness during ham consumption were again linked to instrumental texture parameters (TPA hardness, TPA chewiness and shear force). The perception of fibrousness was related to the degree of fragmentation of the ham in the mouth, and juiciness seemed to be related to the amount of saliva taken up by the product.

Relating texture perception of cooked ham to the bolus evolution in the mouth

A. Rizo[1], E. Peña[2], A.D. Alarcon-Rojo[2], S. Fiszman[1] and A. Tarrega[1]*

[1]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC). Agustín Escardino, 7. Paterna (Valencia), Spain

[2]Departamento de Tecnología de Productos de Origen Animal (TPOA), Universidad Autónoma de Chihuahua, Chihuahua, México

*Corresponding author's email: atarrega@iata.csic.es

Key words: Cooked ham; Texture; Bolus; Oral Processing

Abstract

The aim of the present study was to characterize the dynamics of the bolus formation that take place during mastication of commercial cooked ham samples. In addition, the relationships between these properties and texture perception were studied. Five commercial samples which presented different mechanical properties and moisture contents were studied. Ten participants were asked to chew the cooked ham samples normally and to expectorate the bolus after different chewing periods. Oral activity measurements (chewing time and number of chewing strokes), moisture content, saliva uptake and particle size distribution in the boluses were obtained. Seventeen participants evaluated the sensory perceptions generated over the sample consumption time, using the Temporal Check-All-That-Apply (TCATA) method. The results revealed that the duration of mastication and number of chewing cycles through to swallowing varied significantly among the cooked ham samples and were mainly related to instrumental texture parameters. The pattern of fragmentation under mastication also varied greatly between samples. Sensations of softness and hardness during ham consumption were again linked to instrumental texture parameters (TPA hardness, TPA chewiness and shear force). The perception of fibrousness was related to the degree of fragmentation of the ham in the mouth, and juiciness seemed to be related to the amount of saliva taken up by the product.

1 Introduction

Eating food involves different oral processes, such as comminution, insalivation, and mixing, to form a well-lubricated and cohesive bolus that can be swallowed safely and comfortably. During this process, food products undergo a series of changes, which determine their further behaviour in the digestive tract. In addition, sensory perception of food occurs during this step. The characterization of bolus properties at different stages of eating or just before swallowing can provide insights into the relation between food structure and the dynamics of texture perception in the mouth (Tournier et al., 2017). These stages/levels of transformation are complex and challenging to capture and measure (Stokes, Boehm, & Baier, 2013); knowledge of them can be useful for designing products with tailored sensory profiles.

Approaches have been developed to analyse the properties of the bolus, obtained either in vivo or in vitro. They are mainly based on measuring bolus water content, bolus rheology, or bolus particle size (Panouillé, Saint-Eve, & Souchon, 2016).

Saliva is essential in food bolus formation, acting as an agent that favours bolus cohesiveness and lubrication, which are key requirements for triggering safe swallowing. The amount of saliva incorporated into the bolus can determine a number of bolus characteristics. The amount of saliva (which is usually measured gravimetrically) varies between individuals and/or according to the food characteristics. Some of these characteristics are the initial water content, water absorption capacity, and mechanical properties of the food (Loret et al., 2011; Mioche, Bourdiol, Monier, & Martin, 2002; Panouillé et al., 2016; Tarrega, Yven, Sémon, & Salles, 2011). Rheology provides information about the mechanical features of the bolus and has been studied in products such as cheese (Tarrega et al., 2011), bread, (Jourdren et al., 2016a), breakfast cereals (Loret et al., 2011) and gels (Devezeaux de Lavergne, van de Velde, van Boekel, & Stieger, 2015a), all of which, during chewing, form a bolus with a paste-like consistency that is suitable for evaluation by rheological techniques.

The study of the breakdown path and fragmentation of the solid food product bolus is also relevant. A variety of methods to measure particle size distribution in the bolus have been applied, including sieving methods (Devezeaux de Lavergne et al., 2015a), laser diffraction (Hwang et al., 2012), and image analysis (Tournier et al., 2017). Particle size characterization of the bolus can provide useful information on the different degrees of in-mouth comminution and agglomeration mechanisms, which are obviously determined by the food characteristics (Jalabert-Malbos, Mishellany-Dutour,

Woda, & Peyron, 2007; Panouillé, 2016). Some studies have been conducted to compare the breakdown patterns and the particle size distribution at the point of swallowing of different food products such as jelly, carrot, cheese, and nuts (Chen, Khandelwal, Liu, & Funami, 2013), bread, cake, peanuts and cheese (Engelen, Fontijn-tekamp, & van der Bilt, 2005), and peanuts, carrots, olives, mushrooms, egg, ham, chicken, cheese, and coconut (Jalabert Malbos et al., 2007). Different fragmentation paths have been observed for different food boluses, which present a wide range of particle size distributions before swallowing. These differences are mainly related to certain characteristics, such as water content, hardness, and other mechanical properties of the different food types.

Texture sensations have usually been related to the initial structure and mechanical properties of food, but in recent years increasing attention has been paid to how this structure is transformed during food mastication and swallowing and during the in-mouth cleaning procedures after swallowing. This knowledge can explain the sensory perception of some specific texture attributes (Foegeding, Vinyard, Essick, Guest, & Campbell, 2015; Morell, Hernando, & Fiszman, 2014). A number of studies analyzed the relationship between sensory perception and oral processing in products such gels (Devezeaux de Lavergne et al., 2015a), bread (Jourdren, Saint-Eve, Panouillé, Lejeune, Déléris, & Souchon, 2016b) and sausages (Devezeaux de Lavergne, Derks, Ketel, de Wijk & Stieger, 2015b) using Temporal Dominance of Sensations (TDS) for measuring sensory perception. This technique registers the sensations that are dominant along the time of product consumption. The TDS sensory trajectories of model gels were related to the properties of bolus at different stages of mastication (Devezeaux de Lavergne et al., 2015a). Firmness sensation, dominant at the beginning of gel consumption, was correlated to bolus hardness and flowability. Elasticity and stickiness, in the middle of eating sequence, were correlated with gel bolus resilience and adhesiveness. At the end, creaminess and graininess were related to bolus flowability and number of particles, respectively. Jourdren et al. (2016b) evaluated in bread the dynamics of texture perception using TDS and Progressive Profiling (PP). Due to the difficulty of using TDS data for regression, authors used data from PP technique to model the relation between the intensity of sensory attributes at three stages of consumption with bolus and bread properties by means of partial least square regression. Temporal Check-All-That-Apply (TCATA) is a recently developed technique for evaluating the sensations perceived during food consumption (Ares et al., 2016). Differently to TDS, it allows

selecting several attributes simultaneously without forcing the subject to select only the dominant one. To our knowledge, there are no studies that relate bolus properties to sensory perception assessed by TCATA.

In the case of meat products like cooked ham, oral processing transformation occurs through the compression and shear forces generated by chewing and biting, leading to the formation of a cohesive bolus where saliva is present along with liquid released from the meat itself (Mioche, Bourdiol, & Monier, 2003; Yven, Culioli, & Mioche, 2005). Texture properties such as juiciness, tenderness and fibrousness are the key attributes which determine meat product quality and acceptability (Krzywdzińska-Bartkowiak, Rezler, & Gajewska-Szczerbal, 2016). These sensory features have been related to the initial structure of the meat tissue (such as connective tissue content and distribution, muscle fibre diameters, etc.) (Wang et al., 2015). Little information is available concerning the bolus properties of meat food products with only a few examples of studies on cooked meat (Mioche et al., 2003; Yven et al., 2005) and on sausages (Devezeaux de Lavergne et al., 2015b). The latter research team also studied the relation with the dynamic texture perception of sausages evaluated by TDS and showed that differences in bolus properties among short and long duration eaters explained their differences in dynamic texture perception towards the end of mastication. Differences in juiciness, stickiness and graininess between the two groups of participants were related to the observed differences in the bolus properties at swallowing. Our hypothesis is that differences in texture perception between different samples of a meat product could also be explained by the properties of bolus during eating.

Thus, it could be of great interest to study a processed meat product such as cooked ham in order to establish the links between in-mouth sensory perceptions and the evolution of properties of the bolus at different mastication stages.

The aims of the present study were 1) to characterize the bolus properties during mastication of commercial cooked ham samples, and, 2) to relate these properties to perceptions of the ham's texture.

2 Materials and methods

2.1 Samples

Five commercial cooked pork ham products (A, B, C, D, and E) were used in the present study. The samples were bought both in block form, for instrumental texture

analysis, and sliced (2 mm thick) for the other analyses. The lists of ingredients on the label of each product are shown in Table 1. Sample A was a cooked pork luncheon meat with 55% pork, whereas the rest of the cooked ham products contained at least 82% pork. The sample E label included the claim "reduced salt content" and the ham contained 21% to 40% less salt than the rest of the samples. The samples were stored at -20 °C and thawed at 4 ºC one day prior to the analyses.

2.2 Instrumental texture of ham samples

Ham sample blocks were cut into cylinders (20-mm height, 20-mm diameter) and kept at 4 ºC for 24 h before applying Texture Profile Analysis (TPA) and Warner-Bratzler shear tests.

Both tests were conducted with a TA-XT.plus Texture Analyser equipped with Texture Exponent version 6.0 software (Stable Microsystems, Godalming, UK). Six replications of both methods were conducted for each sample.

TPA was performed using a 75-mm diameter flat aluminium disk probe (SMS P/75) at a test speed of 1 mm/s up to 20 % compression of the initial height. From the force-time curves, hardness (N) and chewiness (N) were obtained.

The Warner-Bratzler blade was used with a slotted blade insert in the HDP/90 Heavy Duty Platform. The samples were cut with the blade perpendicular to the muscle fibres, using a speed of 2 mm/s and a blade displacement of 40 mm in order to cut all the way through the sample. The shear force was determined as the maximum force (N).

2.3 Moisture content of ham samples

The moisture content of the samples was determined by a gravimetric method, according to standard 950.46 to determine moisture in meat (AOAC, 1997). Approximately 5 g of previously crushed sample were weighed, thoroughly mixed with 10 g of sea sand and dried to a constant weight in an oven at 105 ºC. The moisture content was expressed as g of water/100g sample (wet basis). The analyses were performed in triplicate on each sample.

2.4 Bolus collection procedures

Ten subjects with good dental health status participated in the mastication experiments. Cooked ham slices (2-mm thick) were cut into rectangular pieces (40 x 10 mm) and rolled up; the weight of the rolls was about 7 ± 1 g, a normal and comfortable portion

size for the participants according to preliminary tests. The samples were identified with random three-digit codes and were served at 4 ºC to the participants. Each participant was asked to place the entire sample in his/her mouth and chew it as usual but without swallowing. The participants were asked to spit the bolus out into a sealable plastic cup after different chewing periods (after 5, 10, and 15 strokes, and after full mastication when the need to swallow was felt).

Over three sessions, one bolus per sample, chewing period and subject was collected for particle size image analysis. The order of sample presentation and the bolus collection time were varied randomly across subjects. The collected boluses were stored at 4 ºC before further analysis.

In a separate session, additional boluses were obtained to determine the amount of saliva uptake after 10 chewing strokes and at the end of the mastication. In this session, oral activity parameters (the chewing time for these two periods and the number of chews to reach complete mastication) were recorded by the researcher. The subjects had a 3 min rest and were provided with still mineral water for rinsing between samples. The 10-stroke and complete chewing rate were calculated.

2.5 Bolus particle characterization by image analysis

The boluses collected for particle analysis were rinsed with distilled water, filtered (73 g/ m^2 filter paper), and the excess of water after rinsing was left to drain 15 minutes to avoid water drops over the scanner glass. After that, bolus particles were spread out on a clean, dry, transparent glass surface (30 x 21 cm). The ham particles were carefully placed horizontally, separate from one another, and were digitized in TIF format at 600 ppi on a scanner (Canon MP270 model K.10339, NY., USA), using a black background. The images of the spread-out bolus particles were analysed using Nis-Elements® BR 3.2 software (Nikon, Tokyo, Japan). For this purpose, the sample images were binarized using a histogram-based segmentation process, according to the predefined intensity threshold value. All the objects were checked and any unsuitable artefact (fibres and particles touching the frame) were excluded from the evaluation. The particle size distribution, number of particles, and median particle area (a50), which is the particle area corresponding to 50% of total area of the bolus particles at the different mastication times were calculated.

2.6 Water content of boluses

The water content of each bolus was determined immediately after collection to prevent evaporation losses. The method employed for this determination was the same as described for the cooked ham samples in section 2.3. The saliva uptake was calculated by subtracting the water content of the ham sample (%) from the water content of the expectorated bolus (%), assuming that water content gain was only due to saliva uptake.

2.7 Sensory evaluation

The sensory perceptions during consumption of the five ham samples were evaluated using the Temporal Check-All-That-Apply (TCATA) method with the fading option. TCATA is a temporal sensory method for characterizing dynamic product properties; the subjects have a list of terms and select the attributes they consider applicable to describe the sensations they perceive (Ares et al., 2016). In the TCATA Fading variant term un-selection is automatic and progressive over a predefined duration of few seconds, so when participants consider that a term is still perceived after it being automatically unselected, they have to select it again. In this work, a fading time of 4 s was employed.

Seventeen subjects (including the ten subjects who participated in the bolus collection experiment) performed the sensory evaluation of the samples. Four training sessions of 30 min each were conducted with the participants to generate and select the most representative sensory attributes and to familiarize them with the methodology. The attributes selected were salty, smoked flavour, ham flavour, soft, hard, fibrous, and juicy. The serving size and sample presentation were as described in section 2.4.

For each sample, the participants registered the sensations perceived during consumption using the TCATA test. They were asked to place the whole sample in their mouths and simultaneously click the “start” button and start chewing. During the consumption time they had to select (and re-select if necessary) all the terms that described the sensations they perceived at each moment of the evaluation and to click the “stop” button when they did not perceive any further sensation. A maximum test duration time of 40 s was established. The time each panellist took to complete the task was recorded for each sample, as well as the duration of each attribute selected during the evaluation. Between samples, the participants were asked to clean their palates with still mineral water. Three replicate evaluations per sample were carried out in three different sessions. The order of sample presentation and the order of attributes in the TCATA list were varied among the subjects, following a Williams Latin square design,

but for each participant the list of attributes was kept in the same order for all the samples. Sensory evaluation took place in standard sensory booths designed in accordance with ISO 8589 (ISO, 2007), under artificial daylight and temperature control (22 °C). The data collection and analysis were carried out using Compusense Cloud (Compusense Inc., Guelph, Canada).

2.8 Data analysis

2.8.1 Instrumental data

One-way analysis of variance (ANOVA) was performed to test for significant differences between the instrumental texture parameters and mean moisture values of the different ham samples. A mixed analysis of variance (ANOVA), considering sample and subject (random), was conducted on the oral activity values (chewing rate and time and number of strokes). A mixed ANOVA considering sample, chewing period and subject (random) and the interaction of sample x chewing was performed on the bolus particle characterization data (number of total particles and median particle area (a50) (area at 50%). The significance of the differences between average values was determined by the Tukey test (α =0.05). Additionally, a principal component analysis (PCA) was run to analyse the variability in instrumental texture parameters of cooked ham samples, oral activity and bolus properties values. These analyses were performed with XLSTAT statistical software (version 2016, Microsoft Excel®, Adinsoft, Paris, France).

2.8.2 Sensory data

The terms each participant selected for each sample throughout the evaluation were obtained every 1 s. For each sample, the aggregated data across all participants and replicates were represented using line plots. The Fisher-Irwin test (Fisher, 1935; Irwin, 1935) was used to assess the differences in attributes between each sample and the average for the rest of the samples. When the attribute citation proportions differed significantly from the other products, the differences were highlighted on the TCATA curves at the relevant time segments. Comparisons were made at two-sided level 5%. The temporal sensory data analyses were carried out using R package version 3.3.2 (R Core Team, 2016) with the tempR package (Castura, 2016). TCATA trajectories were obtained by applying Correspondance Analysis to citation frequency of an attribute per

sample at specific time slices (every 4 s). XLSTAT statistical software (version 2016, Microsoft Excel®, Adinsoft, Paris, France) was used.

3. Results and discussion

3.1 Initial sample characterization

As shown in Table 2, the products presented significant (α=0.05) differences in mean instrumental texture parameter and water content values. Sample B exhibited higher hardness, chewiness and shear force values than the rest of the samples, whereas samples A and E showed the lowest values for these parameters. Sample A presented the lowest water content and samples D and E the highest amount of water.

3.2. Oral activity and water content

The times and chewing rates during the two mastication tasks (at 10 strokes and at end of mastication) in Table 3. The chewing rate for the 10-strokes task varied significantly among samples (F=3.75, p=0.012) and was significantly lower for sample D than for the rest, indicating that at the beginning of mastication the participants chewed this sample more slowly than the others, probably due to more difficult or complex management of this sample in the mouth. However, the chewing rate for the total period of consumption did not very significantly among the samples (F= 0.60, p=0.67), indicating that in general the participants masticated the samples at a similar rate, independently of their texture.

The duration and number of strokes until swallowing varied significantly among the ham samples (F= 4.62, p=0.004 and F=4.13, p=0.007, respectively). A shorter mastication time and a lower number of chewing strokes were observed for samples A and E, which exhibited the lowest values for TPA hardness and chewiness and for shear force. Samples D and B displayed longer chewing times and numbers of strokes, although the values were only significantly higher than the rest for sample D, and not for sample B despite its being the hardest sample according to the instrumental texture tests. The influence of hardness on oral activity parameters such as masticatory duration and strokes has been described previously in the literature (Çakir et al., 2012; Foegeding et al., 2015; Jalabert Malbos et al., 2007; Peyron, Lassauzay, & Woda, 2002).

The water content of the boluses increased during chewing, as expected, and in general, at the end of mastication all the boluses had reached similar moisture values (78-80%), which indicates that in order to form a cohesive and swallowable bolus, insalivation compensated in part for the initial differences in water content of the ham samples. Similarly, Loret et al. (2011) found that the different initial water content of breakfast cereals did not influence the amount of moisture at the swallowing point. In the present study, the changes in water content were attributed to saliva incorporation, although according to some authors some juice released from the meat matrix could also be considered (Mioche et al., 2002). Sample A registered a significantly larger amount of saliva incorporation in the bolus at the point of swallowing compared with the other samples, probably because of differences in composition. Product A, commercially known as "pork luncheon meat", contains 55% pork meat and potato starch, among other ingredients, whereas the rest of the cooked ham products evaluated in the present study had a pork content of at least 82% and no addition of starch (Table 1). The fact that more saliva was retained by sample A could be explained by the water absorption capacity of the starch, which, indeed, is used by the industry to increase water retention in meat products (Toldrá, Mora, & Flores, 2010).

3.3. Particle size distribution in bolus obtained *in vivo*

During chewing, the ham material was fragmented until it was transformed into a bolus ready to swallow. In order to characterize this fragmentation, the changes in the size and number of particles were studied by analysing the scanned images. As an example, Figure 1 shows the binarized images of sample C and D boluses expectorated by one of the participants after 5 and 15 chewing strokes. From each image, the percentage of the area occupied by particles of different size ranges was calculated (Figure 2). As expected, the percentage of large particles decreased and the percentage of small particles increased over the chewing process. In general, at 5 strokes the boluses mainly presented particles larger than 100 mm^2. At the end of mastication, particle sizes between 10 and 50 mm^2 constituted the most abundant fraction. The pattern of particle size reduction during chewing varied among the samples. Sample C presented the highest number of small particles (1-50mm^2) from the beginning, and a rapid and intense fragmentation as chewing progressed. Samples D and E presented slower fragmentation, as the percentage of large particles remained higher than for the rest of the samples. At the swallowing point, all the boluses presented under 18% of the area

occupied by big particles (>100 mm^2) and at least 55% of the area occupied by particles smaller than 50 mm^2. To compare the degree of fragmentation among samples, the number of ham particles and the median particle area (a50, in mm^2) were obtained (Figure 3). In the literature, the particle size distribution has commonly been shown as the median particle diameter (d50, in mm, which corresponds to the length at 50% of cumulative mass) when measurements have been performed by sieving. In the present study, image analysis gave the particle size in units of area, and the median area (a50) is the particle area corresponding to 50% of cumulative area. Both number of particles and median particle area (a50) significantly varied between samples ($F=6.4$, $p<0.001$ and $F=28.60$, $p<0.001$ respectively) and chewing strokes ($F=23.8$, $p<0.001$ and $F=254.7$, $p<0.001$, respectively). As expected, the number of particles in the bolus increased with the number of chewing strokes. The sample C boluses showed the highest number of particles in all the chewing periods, indicating a higher degree of fragmentation. Likewise, product A also exhibited an intense and rapid fragmentation pattern, but only at the initial chewing times (5 and 10 strokes), after which it slowed down significantly. The number of particles was lower for sample D, especially at the beginning of consumption (5 and 10 strokes).

The median particle area found in the boluses was lower for sample C, which is consistent with a high degree of fragmentation. The particles in the sample D and E boluses presented higher median area (a50) values, indicating that there was less fragmentation of these samples, and even at the swallowing point the particles were bigger than in the rest of the samples. Previous studies comparing different food categories have shown that differences in bolus particle size at the swallowing point are mainly related to differences in the hardness of the food product, with harder foods presenting smaller particles (Peyron et al., 2002, Jalabert-Malbos et al., 2007; Chen et al., 2013). In the present study, food samples from the same category were compared and the differences in particle size distribution in the boluses at swallowing point, as observed in Figure 2, were not related to the hardness of the samples but to the fragmentation properties of the product itself. The same pattern was observed when considering the median particle area values of the boluses at swallowing point, although the differences among samples were not significant ($\alpha=0.05$).

A PCA was carried out to summarize the variations between samples regarding their instrumental texture, oral activity and bolus particle size distribution and moisture (Figure 4). The two first components explained 81.6% of the variability. First

component separates sample C on the left, because presented bolus with high number of particles of small size and sample D on the right that presented bolus with lower number of particles and bigger size. The second component separates on the top sample B (with high hardness, chewing and shear force values) that needed more chewing strokes and a longer chewing time, from samples A and E with low values for these parameters. Sample A on the down left side of the map was differentiated from the rest of the samples as it presented a higher saliva uptake.

3.4 Sensory sensations perceived during ham consumption

The TCATA curves, presented in Figure 5, show the proportion of citations that each attribute received at each moment of consumption of each ham sample. The temporal profiles were very different among samples. A highlighted line (thicker trace) indicates that for this sample the proportion of citations of the attribute differed significantly (higher or lower) from its average of citations for the rest of the samples. Sample A was mainly characterised by the attributes *soft* and *juicy*, which appeared at the beginning of consumption, with maximum citation frequencies at 5 s and 8 s, respectively. During most of the consumption time, the citation frequencies of these two attributes were significantly higher for sample A than for the average of the rest of samples. *Salty taste* appeared later in this sample and its maximum citation frequency was reached at 11 s, at which point it was significantly more cited than for the other samples. In contrast, *hardness* was the sensation used to describe sample B, with maximum citation at 5 s although throughout the consumption time it was significantly higher for this sample than for the rest. *Fibrous* and *smoked flavour* were the most representative attributes in the sensory perception of sample C. *Fibrous* appeared sooner, reached the maximum citation frequency at 7 s and started to decrease after 16 s, while *smoked flavour* reached the maximum citation frequency at 11 s and started to decrease after 23 s. For sample D the attribute curves were quite flat, with no salient attributes except for *saltiness*, the most-cited attribute from 9 to 30 s. Finally, for sample E the sensation of *softness* was perceived almost from the beginning (maximum citation frequency at 6 s) and its citation frequencies were significantly higher than the average for the rest of the samples. *Ham flavour* was also relevant for this sample, with a maximum citation frequency at 10 s and significantly higher citation rates than for the rest of the samples.

TCATA trajectories summarizes the dynamic profile of the cooked ham samples (Figure 6). The two first dimensions explained 74.6% of the total inertia. The first

factor explained mainly differences among samples. It separates samples A and E (left side) described as soft and juicy from samples B and C (right side) being described as hard, fibrous and with smoked flavour. Differences with time were mainly explained by the second factor. In general, the trajectory of each sample started on the top of the map with higher citation proportions of texture sensations (soft for sample A and E, hard for sample B and fibrous for C), progressing to flavour attributes at the end (ham flavour and salty for sample A, B, and D and smoke flavour for samples B and C). The length of trajectories also varied among samples. Sample B exhibited a long trajectory that started far from the rest and far from its end, because it was the only sample eliciting hardness sensation which decreased over time. On the contrary, samples D and E presented shorter and more circular trajectories as sensations registered did not much differed along product consumption time.

The TCATA curves could also be expected to provide information on how similarities and differences among sample sensory attributes evolved over the consumption time. However, in the present case the perceptions of the sensory sensations elicited by the cooked ham were not strongly time-dependent. Especially in the case of texture, the attributes that characterised a sample remained the same over most of the consumption period. The low temporal complexity registered in this case could be due to the nature of the product, which is indeed quite neutral, with no salient attributes and no marked changes, or to the nature of the TCATA technique and the type of data collected. For instance, an attribute cited by all the participants, but at different times, could be overlooked or not be considered. This could be the case of the flavour attributes (except *smoked* flavour), where the sensations were generally of short duration and the participants did not select the same attribute at the same time, resulting in flat curves.

3.5 Relation between texture sensations, bolus properties and oral activity during cooked ham consumption

As commented above, each sample elicited one or two texture sensations that clearly differentiated it from the rest. It could be interesting to analyse which of the samples' properties are related to these sensations and how they changed during consumption.

Softness was mainly associated with low instrumental hardness, chewiness and shear force values. The participants perceived a soft sensation during consumption of samples A and E, which were those with the lowest values for these instrumental parameters. Additionally, the oral activity of the participants seemed to be related to the *softness*

sensation, as these two samples showed shorter chewing times and a lower number of chewing strokes over the consumption time.

Hardness was associated with high instrumental hardness, chewiness and shear force values. The participants perceived hardness only in sample B, which presented higher values for these parameters. Oral activity was lengthy not only for sample B but also for sample D. Sample D was not perceived as hard, but it showed a low fragmentation rate and probably needed more chewing time and a greater number of strokes to achieve an adequate particle size for swallowing. The chewing time seems to have been related to the hardness of the cooked ham, and also to the breakdown pattern. As Chen et al. (2013) stated different factors are involved in determining a ready-to-swallow bolus: food particle size, texture, the deformability of the food particles, and the amount of fluid (saliva) within the bolus cluster.

Fibrousness concerns the in-mouth perception of ham muscle fibres (Guàrdia, Aguiar, Claret, Arnau, & Guerrero, 2010). From the results of the present study, it seems to be linked to a high degree of fragmentation of ham during chewing. The subjects perceived fibrousness during most of the consumption time of sample C, which showed the highest degree of fragmentation (the highest number of particles). Fibrousness was also significant at certain points of consumption for sample B, which also showed a high degree of fragmentation.

Juiciness initially seemed not to be related to the amount of water in the ham sample, because the juiciness sensation was mainly perceived during consumption of sample A, which presented the lowest initial water content. However, this sample showed the highest saliva uptake values, so it is possible that juiciness may be related to the ability of the ham to retain or incorporate liquid during chewing. Considering the other four samples, the frequency of *juiciness* citation was lower during consumption of samples B and C, which contained a lower amount of water, than it was for samples D and E, which had a higher water content.

It should be mentioned that the present study is merely an attempt to establish relationships between texture sensations and bolus properties. A statistical analysis could not be applied because of the different procedure in data collection (time vs number of strokes) which is limitation of the study. Additionally, the fact that samples in the study are commercial products, together with the low number of samples, limits the possibility of arriving at stronger associations or conclusions. In commercial cooked ham samples, many factors could affect the product properties (raw meat, brine

composition, mechanical treatment and cooking/cooling treatment, among others) (Delahunty, McCord, O'Neill, & Morrissey, 1997; Müller, 1989), and therefore the results. Further investigation with a higher number of experimental samples with controlled variations would allow more robust conclusions to be reached.

4. Conclusions

The dynamics of bolus formation and sensory perception during consumption of commercial cooked ham products were characterized. Measuring the particle size distribution of *in vivo* boluses at different chewing times provides valuable information about the fragmentation patterns of cooked ham in the mouth, which differed among commercial products.

Sensory perceptions of *softness/ hardness* during ham consumption were related to low/high values, respectively, of instrumental texture parameters (TPA hardness, TPA chewiness, shear force). The perception of *fibrousness* was related to the degree of fragmentation of the ham in the mouth. *Juiciness* seemed to be related both to the amount of saliva incorporated into the bolus and to the initial water content of the product.

The present study is a first attempt to relate dynamically assessed texture sensations elicited by cooked ham products with the properties of the bolus formed during their consumption.

Acknowledgements

To the Spanish Ministry of the Economy and Competitiveness for financial support (project AGL-2016-75403-R) and for the Ramon y Cajal contract to author Tarrega (support of EU FEDER funds). To Generalitat Valenciana (Project Prometeo 2017/189). To CONACYT of Mexico (fellowship # 214894) and to the Innovatec project (# 233472) for funding the stage of author Peña. To Jose Coll for technical advice on image analysis. To Mary Georgina Hardinge for assistance in correcting the English manuscript.

References

Association of Official Analytical Chemists (AOAC, 1997). (16th ed.). Washington: *Official methods of analysis*

Ares, G., Castura, J. C., Antúnez, L., Vidal, L., Giménez, A., Coste, B., & Jaeger, S. R. (2016). Comparison of two TCATA variants for dynamic sensory characterization of food products. *Food Quality and Preference, 54*, 160–172.

Çakir, E., KoÇ, H., Vinyard, C. J., Essick, G., Daubert, C. R., Drake, M., & Foegeding, E. A. (2012). Evaluation of texture changes due to compositional differences using oral processing. *Journal of Texture Studies, 43*(4), 257–267.

Castura, J. C. (2016). tempR: Temporal Sensory Data Analysis. R package version 0.9.9.10. https://cran.r-project.org/web/packages/tempR/index.html [Accessed 4 September, 2017]

Chen, J., Khandelwal, N., Liu, Z., & Funami, T. (2013). Influences of food hardness on the particle size distribution of food boluses. *Archives of Oral Biology, 58*(3), 293–298.

Delahunty, C. M., McCord, A., O'Neill, E. E., & Morrissey, P. A. (1997). Sensory characterisation of cooked hams by untrained consumers using free-choice profiling. *Food Quality and Preference, 8*(5–6), 381–388.

Devezeaux de Lavergne, M., van de Velde, F., van Boekel, M. A. J. S., & Stieger, M. (2015a). Dynamic texture perception and oral processing of semi-solid food gels: Part 2: Impact of breakdown behaviour on bolus properties and dynamic texture perception. *Food Hydrocolloids, 49*, 61–72.

Devezeaux de Lavergne, M., Derks, J. A. M., Ketel, E. C., de Wijk, R. A., & Stieger, M. (2015b). Eating behaviour explains differences between individuals in dynamic texture perception of sausages. *Food Quality and Preference, 41*, 189–200.

Engelen, L., Fontijn-tekamp, A., & van der Bilt, A. (2005). The influence of product and oral characteristics on swallowing. *Archives of Oral Biology, 50*, 739–746.

Fisher, R. A. (1935). The logic of inductive inference. *Journal of the Royal Statistical Society, 98*, 39–54.

Foegeding, E. A., Vinyard, C. J., Essick, G., Guest, S., & Campbell, C. (2015). Transforming Structural Breakdown into Sensory Perception of Texture. *Journal of Texture Studies, 46*(3), 152–170.

Guàrdia, M. D., Aguiar, A. P. S., Claret, A., Arnau, J., & Guerrero, L. (2010). Sensory characterization of dry-cured ham using free-choice profiling. *Food Quality and Preference, 21*(1), 148–155.

Hwang, J., Kim, D. K., Bae, J. H., Kang, S. H., Kim, B. K., Kim, B. K., & Lee, S. Y. (2012). The effect of rheological properties of foods on bolus characteristics after mastication. *Annals of Rehabilitation Medicine, 36*(6), 776–784.

Irwin, J. O. (1935). Tests of significance for differences between percentages based on small numbers. *Metron, 12*, 83–94.

ISO (2007). Sensory analysis: General guidance for the design of test rooms. ISO standard 8589. Geneva, Switzerland: International Organization for Standardization

Jalabert-Malbos, M. L., Mishellany-Dutour, A., Woda, A., & Peyron, M. A. (2007). Particle size distribution in the food bolus after mastication of natural foods. *Food Quality and Preference, 18*(5), 803–812.

Jourdren, S., Panouillé, M., Saint-eve, A., Déléris, I., Forest, D., Lejeune, P., & Souchon, I. (2016a). Breakdown pathways during oral processing of different breads: impact of crumb and crust structures. *Food & Function, 7*, 1446–1457.

Jourdren, S., Saint-Eve, A., Panouillé, M., Lejeune, P., Déléris, I., & Souchon, I. (2016b). Respective impact of bread structure and oral processing on dynamic texture perceptions through statistical multiblock analysis. *Food Research International, 87*, 142–151.

Krzywdzińska-Bartkowiak, M., Rezler, R., & Gajewska-Szczerbal, H. (2016). The influence of meat muscle structural properties on mechanical and texture parameters of canned ham. *Journal of Food Engineering, 181*, 1–9.

Loret, C., Walter, M., Pineau, N., Peyron, M. A., Hartmann, C., & Martin, N. (2011). Physical and related sensory properties of a swallowable bolus. *Physiology and Behavior, 104*(5), 855–864.

Mioche, L., Bourdiol, P., Monier, S., & Martin, J. F. (2002). The relationship between chewing activity and food bolus properties obtained from different meat textures. *Food Quality and Preference, 13*(7–8), 583–588.

Mioche, L., Bourdiol, P., & Monier, S. (2003). Chewing behaviour and bolus formation during mastication of meat with different textures. *Archives of Oral Biology, 48*(3), 193–200.

Morell, P., Hernando, I., & Fiszman, S. M. (2014). Understanding the relevance of in-mouth food processing. A review of invitro techniques. *Trends in Food Science and Technology, 35*(1), 18–31.

Müller W.D., (1989). The technology of cooked cured products. *Fleichwirtschaft, 69*, 1524–1528

Panouillé, M., Saint-Eve, A., & Souchon, I. (2016). Instrumental methods for bolus characterization during oral processing to understand food perceptions. *Current Opinion in Food Science, 9,* 42–49.

Peyron, M. A., Lassauzay, C., & Woda, A. (2002). Effects of increased hardness on jaw movement and muscle activity during chewing of visco-elastic model foods. *Experimental Brain Research, 142*(1), 41–51.

Stokes, J. R., Boehm, M. W., & Baier, S. K. (2013). Oral processing, texture and mouthfeel: From rheology to tribology and beyond. *Current Opinion in Colloid and Interface Science, 18*(4), 349–359.

Tarrega, A., Yven, C., Sémon, E., & Salles, C. (2011). In-mouth aroma compound release during cheese consumption: Relationship with food bolus formation. *International Dairy Journal, 21*(5), 358–364.

Toldrá, F., Mora, L., Flores, M., (2010). Cooked ham. In: Toldrá, F. (Ed.), *Handbook of Meat Processing*. Wiley-Blackwell, Ames, IA, pp. 299–311.

Tournier, C., Devezeaux de Lavergne, M., van de Velde, F., Stieger, M., Salles, C., & Bertrand, D. (2017). Investigation of oral gels breakdown using image analysis. *Food Hydrocolloids, 63*, 67–76.

Wang, X., Sun, Y., Liu, A., Wang, X., Gao, J., Fan, X., Shang, J., Wang, Y. (2015). Modeling structural and compositional changes of beef during human chewing process. *LWT - Food Science and Technology, 60*(2), 1219–1225.

Yven, C., Culioli, J., & Mioche, L. (2005). Meat bolus properties in relation with meat texture and chewing context. *Meat Science, 70*(2), 365–371.

Table 1. Ingredients and nutritional facts about the cooked ham products

Sample	Ingredients	Nutritional composition (g/100g)			
		Protein	**Fat**	**Carbohydrate**	**Salt**
A	Pork (55%), water, potato starch, salt, milk protein, soy protein, sugar, lactose, corn dextrose, stabilisers (sodium triphosphate, carrageenan), flavourings, flavour enhancer (monosodium glutamate), preservatives (sodium nitrite, antioxidants (sodium erythorbate), colourings (carminic acid).	13	2	9	2
B	Pork (85%), water, salt, lactose, corn dextrose, sugar, spices, stabilisers (sodium triphosphate, carrageenan), flavour enhancer (monosodium glutamate), antioxidants (sodium erythorbate, sodium citrate), preservatives (sodium nitrite).	16.5	1.5	2	2.5
C	Pork (82%), water, salt, corn maltodextrin, lactose, sugar, corn dextrin, flavourings, smoke flavouring, stabilisers (sodium triphosphate, sorbitol, carrageenan), antioxidants (sodium erythorbate, sodium citrate), preservatives (sodium nitrite).	15.5	1.5	2	2
D	Pork (85%), water, salt, dextrose, stabilisers (sodium triphosphate, carrageenan), antioxidants (sodium ascorbate), spices, flavourings, flavour enhancer (monosodium glutamate) preservatives (sodium nitrite).	18.6	2.5	0.9	1.9
E	Pork (85%), water, dextrose, sugar, potassium chloride, sodium chloride, stabilisers (sodium triphosphate, carrageenan, sorbitol), antioxidants (sodium ascorbate, sodium citrate,), preservatives (sodium nitrite).	18.6	2.5	1.1	1.5

Table 2. Instrumental texture parameters and moisture content of cooked ham samples

Sample	Hardness (N)	Chewiness (N)	Shear force* (N)	Water content (g/100g)
A	7.23 c	7.11 c	7.48 c	71.73 c
B	12.17 a	11.96 a	16.68 a	74.14 b
C	8.38 bc	8.17 bc	12.01 b	73.36 b
D	9.32 b	8.80 b	11.15 b	76.21 a
E	7.31 c	7.21 c	7.43 c	75.65 a
p value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*

Mean values in the same column that do not share letters are significantly different (α=0.05) according to Tukey´s test. Different letters indicate significant differences according to Tukey´s test (α= 0.05).

* Warner Bratzler test

Table 3. Oral activity (chewing rate, time, number of strokes) and bolus moisture (water content and saliva uptake) when eating cooked ham samples.

Sample	Oral activity				Bolus moisture		
	10-strokes chewing rate (chews/s)	Total chewing rate (chews/s)	Total chewing time (s)	Total chewing strokes	10-strokes water content (g/100g)	Total water content (g/100g)	Saliva uptake (g/100g)
A	1.2 ab	1.3 a	15.0 b	19.3 b	76.8 b	78.9 ab	7.2 c
B	1.3 a	1.3 a	18.4 ab	22.7 ab	76.4 b	78.9 ab	4.7 b
C	1.3 a	1.3 a	16.4 ab	20.0 ab	75.9 b	77.9 b	4.6 b
D	1.1 b	1.2 a	20.0 a	23.7 a	78.3 a	80.3 a	4.1 ab
E	1.3 a	1.3 a	14.8 b	18.8 b	76.3 b	78.5 b	2.9 a

Letters indicate significant differences. For each parameter, values that do not share letters are significantly different according to Tukey´s test (α= 0.05).

Figure1

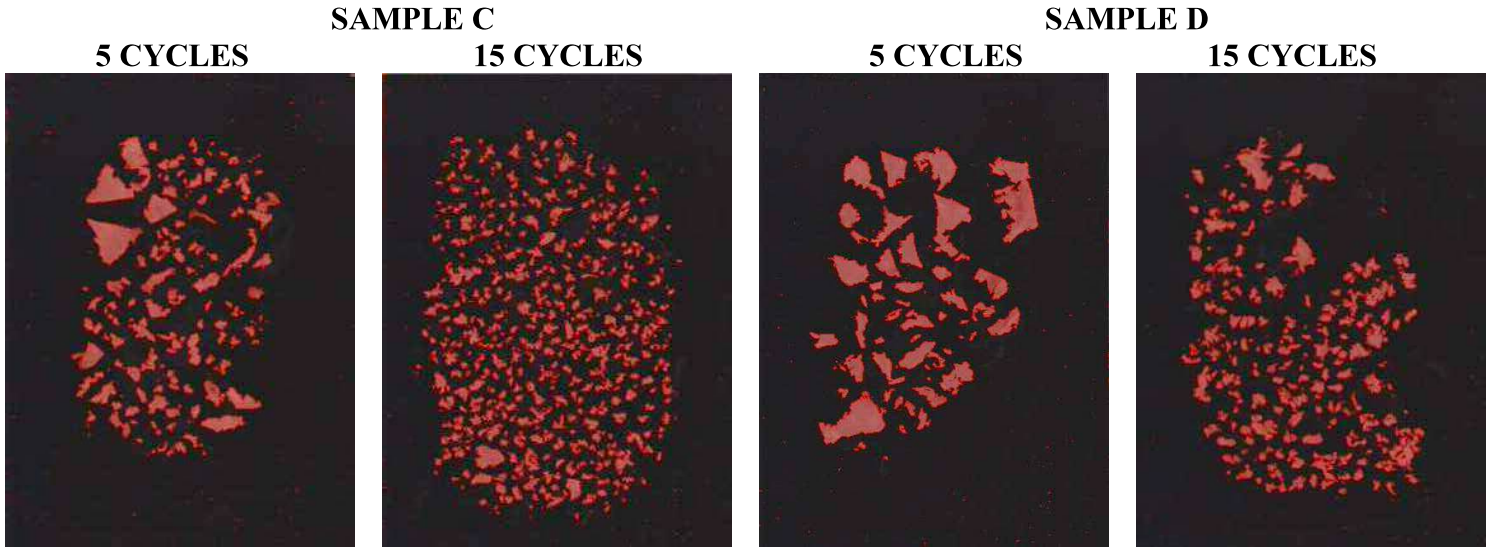

Figure 1. Images of scanned boluses corresponding to samples C and D after 5 and 15 chewing strokes, obtained in vivo from one of the subjects.

Figure2

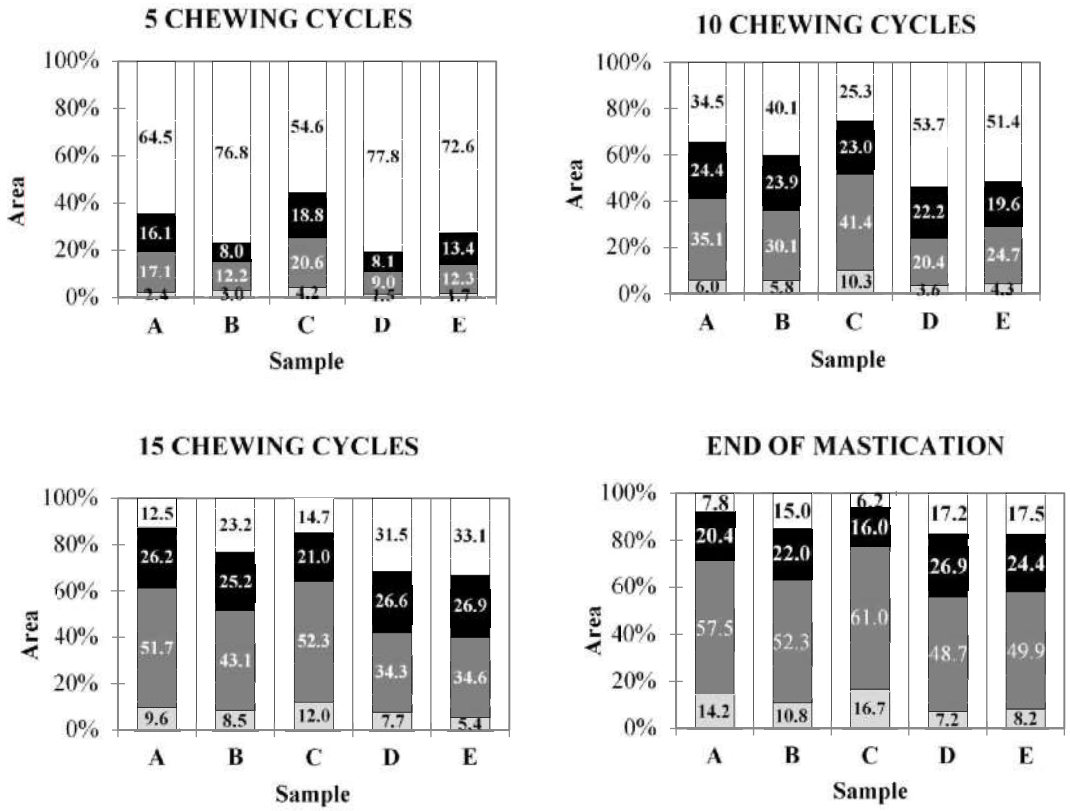

Figure 2 Percentage of area occupied by particles of size (1-10 ■, 10-50 ■, 50-100 ■, and >100mm^2 □) in the cooked ham bolus samples obtained *in vivo* after 5, 10, 15 chewing cycles and at the end of mastication.

Figure3

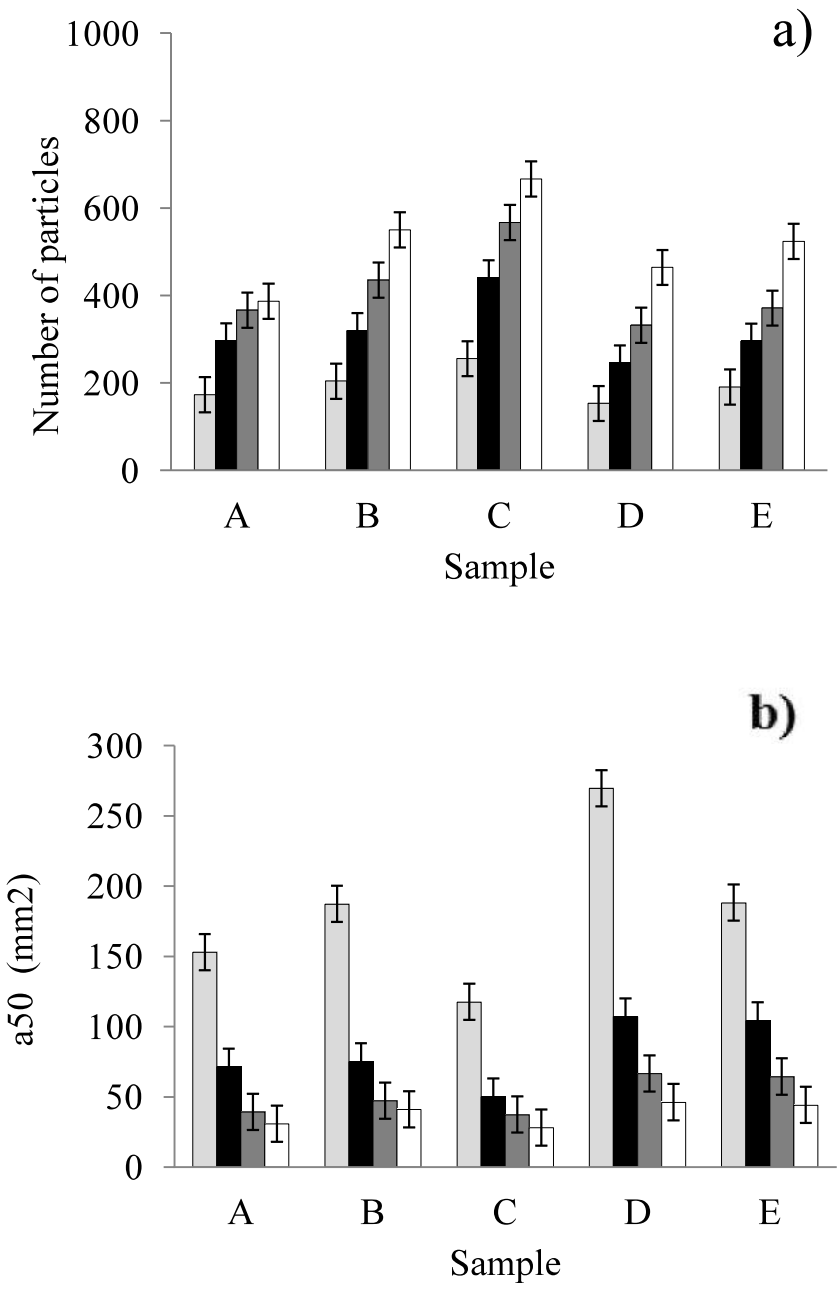

Figure 3. Number of particles (a), and median particle area (a50) (b) of cooked ham boluses obtained in vivo after 5 (■), 10 (■), 15 (■) chewing cycles and at the end of mastication (□). Error bars indicate the HSD interval according to Tukey´s test (α = 0.05).

Figure4

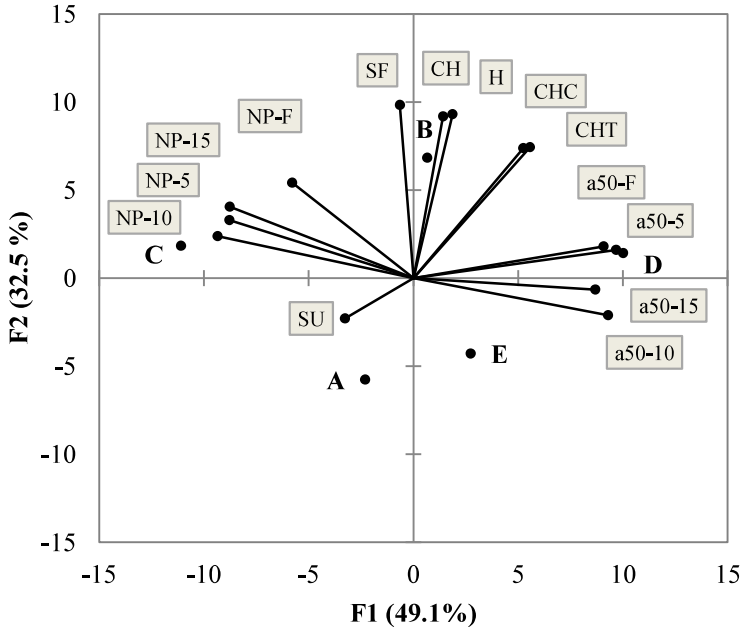

Figure 4. PCA plot showing variation in cooked ham products regarding instrumental texture, oral activity and bolus particle size and saliva uptake.
Hardness: H, chewiness: CH, shear force :SF, number of particles at 5, 10, 15 chewing cycles after full mastication: NP-5, NP-10, NP-15, NP-F respectively, median particle area at 5, 10, 15 chewing cycles and after full mastication: a50-5, a50-10, a50-15, a50-F respectively, saliva uptake after full mastication: SU, chewing cycles after full mastication: CHC, total chewing time: CHT.

Figure5

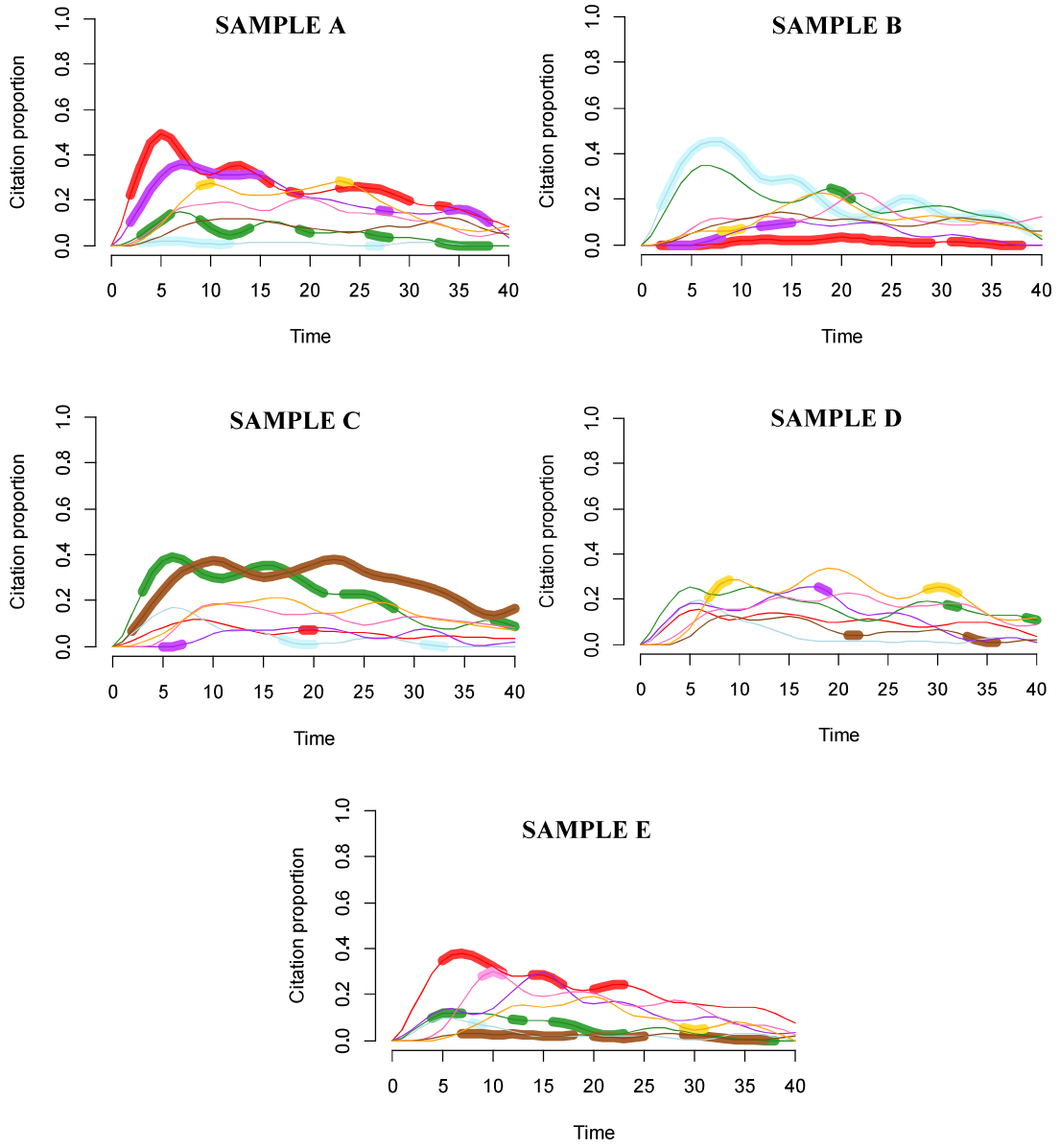

Figure 5. TCATA curves of the attributes (soft —, hard —, juicy —, fibrous —, ham flavour —, smoked flavour —, salty flavour —). Highlighted sections indicate significant differences in the proportion of citations of the attribute at that evaluation time compared to the mean of the values of the rest of the samples. Time is expressed in seconds.

Figure 6

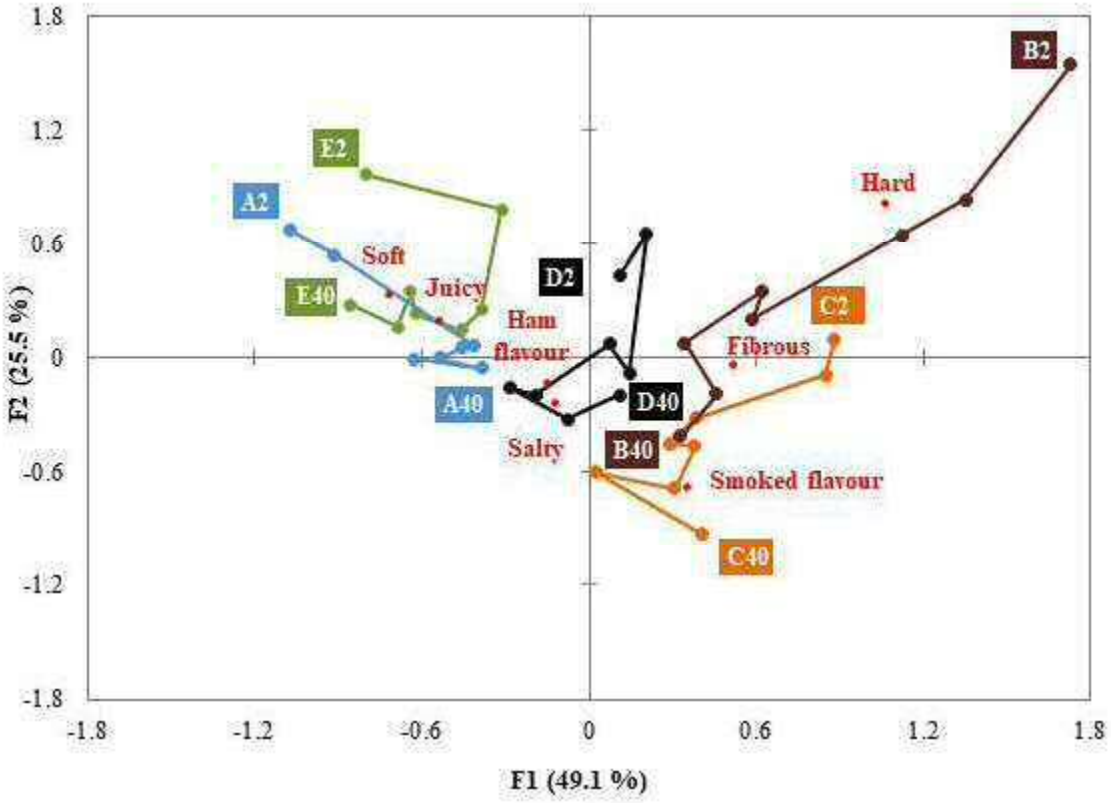

Figure 6. Sensory trajectories of cooked ham samples (A to E) from 2s to 40s obtained from Correspondance Analisys of TCATA data.