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Effect of Water, Sodium Chloride, Lactic Acid, Sodium Nitrite, Sodium Ascorbate and Paprika upon Lightness (L*) in a Dry-cured Sausages Model System

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Abstract: Dry-cured meat products are traditional to Mediterranean countries, but in color science few papers have been published about objective colour measurements. Colour co-ordinates are related to different chemical and physical properties. Color studies during earlier stages can improve technological processes. The effect of the mincing process, paprika and curing agents, during the mixing-resting stage, on the lightness of the dry-cured sausage meat batter, was studied. A dry-cured sausage model system was elaborated. Traditional dry-cured spanish sausage formula was prepared with pork lean meat, spices and curing agents. Lightness determinations were made using CIELAB colour space (illuminant D_{65} and 10° observer). American Meat Science Association Guidelines for colour measurements was followed. Lightness increased with the mincing process and water addition. Significant differences between water and salt addtion were found ($p<0.05$), salt effect compensates mincing and water addition effect upon meat batter lightness. The effect of the salt on L* occurred at the moment of its being added. The sodium nitrite and sodium ascorbate did not change the L*. Paprika addition reduces L* and its effects did not change during the process under study.

Key words: Paprika, lightness, dry-cured sausage, salt, nitrite, ascorbate, lactic acid

INTRODUCTION

Generally, the colour of foodstuffs and meat products in particular, is a determining factor in their selection and acceptance by the consumer[1] .

Meat colour, from a physical point of view, is considered as a surface phenomenon of an opaque solid, where light falling upon it may undergo processes of absorption, reflection or scattering but, where generally there is little transmission[2].

From a chemical point of view, meat colour is the result of the relationship between the different states of the myoglobins (the states of the heme group iron oxidation and type of substituent that occupies sixth position of that group)[3,4].

Measuring meat colour involves two basic methods: human visual appreciation (subjective method) and instrumental analysis (objective method)[5].

Objective determination of colour by means of reflectance spectrophotometry is one of the most commonly used methods owing to its close correlation with the visual perception of the human eye. In contrast to absorption spectrophotometry, reflectance is measured over the surface of the object, thus making destruction of the object unnecessary and allowing the changes of colour over a period of time on the same sample to be evaluated[2,6].

The physical structure of the object, as well as the chemical nature of its components, affects reflectance[7-9]. Sayas-Barberá,[10] reported that in dry-cured meat products color, meat ultrastructure can modified light properties. The reflected light that comes off the object is a visual stimulus and is that which is used to carry out an objective measuring of colour[11].

The International Commission of Illumination (Commission International de l'Eclairage - CIE) has defined the most important and most widely used system today for colour description, which is based on the use of observers and sources of standard illumination[1,12]. The system obtains the CIE tristimuli values based on the visible spectrum, so defining three primary colours: red (X), green (Y) and blue (Z). From those the colour coordinates L* (lightness), a* (red-green), b* (yellow-blue) are mathematically calculated for the CIELAB colour space[12,13].

Measurement of colour by objective methods as CIELAB, is one of the methods used to evaluate shortcomings in meat quality (e.g. PSE or DFD defect)[14].

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Lightness has been used to measure some meat characteristics as water holding capacity[11], fat content[15], moisture content, etc. This color co-ordinate also has been related to myoglobin concentration[16] and defective meat from gallinaceous poultry[17].

The technological processes applied to the foodstuffs, in particular size reduction (comminution)[3,15,18] and mixing techniques, as well as adding additives[19,20], unusual ingredients like dietary citrus fibres[21] and spices[22], affect colour properties[12,23].

During the manufacture of fermented dry-cured meat products a decrease in pH values takes place as a result of microbial metabolic activity[24,27]. The depth light of penetration and reflection are pH dependent. Thus, the low pH causes more light scattering, also increasing reflectance[20] and the high pH less[28].

During the manufacture of Spanish dry-cured sausages, the raw materials are comminuted and then mixed with additives and spices to make up a filling, which is generally left to rest for 12 h, after which it is stuffed, fermented and matured.

In studies carried out upon colour changes during the dry-cured sausage making process, variations have been observed in the lightness of the meat filling during that rest period, but the way in which those changes develop during that period has yet to be established[26,29].

The general aim of this study was to study the evolution of lightness in the minced lean pork as a result of the effect of the mincing process and of the additives used in curing it during the rest period, prior to the sausage stuffing stage.

Particular aims were to study the influence of the mincing process and of adding water -used as a vehicle for the additives and spices- on the lean meat L*; to evaluate the effect of salt (NaCl), sodium nitrite, sodium ascorbate and paprika on seasoned meat L*; to study the effect that pH decrease, through lactic acid activity, has on minced meat L* and on the L* of minced meat with paprika and to understand the evolution of L* of minced meat and seasoned meat during the sausage filling rest period.

MATERIALS AND METHODS

Raw material: Lean meat from three de-boned pig shoulders was used. The meat underwent a 24 h hanging-up period after slaughter. The additives used were of food grade, apart from the lactic acid and salt, which were of analysis grade.

Preparation of the samples: On each shoulder (whole lean meat) nine colour and pH determinations were made (pre-treatment samples, time -1). They were then immediately prepared and processed separately, cutting them into cubes (approximately 10x10 cm). Those sections were then introduced into a meat mincer (Castellvall PTI/106, Castellar del Valles, Spain) equipped with two plates, one with three holes and the other with a 20 cm hole.

Table 1: Amount (%) of additives, paprika and lactic acid added to the minced lean meat in each of the treatments

Treatments	(%) Peecentage
Minced lean meat (control)	-
Water	5.0
Salt (NaCl)	2.306
Sodium Nitrite	0.010
Lactic acid	0.5
Sodium ascorbate	0.050
Paprika	2.5
Sodium Nitrite + salt	0.010 + 2.306
Sodium ascorbate + sodium nitrite + salt	0.050 + 0.010 + 2.306
Paprika + lactic acid	2.5 + 5.0
Sodium ascorbate + sodium nitrite + salt+paprika	0.050 + 0.010 + 2.306 + 2.5

The minced meat obtained from each of the shoulders was divided into eleven 400 g portions. One of the portions was used as a control (minced lean meat without additives) and the ten remaining ones had either water or additives added to them, all of which are shown in Table 1. Those additives and water correspond to quantities used in a standard formula.

To add the additives, paprika and lactic acid of each treatment, they were first dissolved in 5% water and the filling was then mixed to ensure correct distribution.

From each of the eight portions, three Petri dishes of 12 cm diameter and 2.5 cm in depth and a 100 mL beaker were filled up. Three lightness determinations were taken from each of the Petri dishes and three pH measurements taken from each beaker.

Those determinations were made immediately after adding the additives (time 0) and over 12 h at intervals of 1 h between each measurement. The time lapse between the lightness determinations on the whole lean meat (time -1) and those made on the minced meat, with or without additives (time 0) was minimal (approximately 5-6 min) and only that needed to carry out the mincing and mixing operations.

Both the Petri dishes and beakers were covered and maintained at 2±1 °C throughout the experiment to prevent the samples from drying out.

Analytical methods: Lightness determinations were made by means of a Minolta CM-2002 (Minolta Camera Co., Osaka, Japan) spectrophotometer (illuminant D_{65} and 10° observer). American Meat Science Association Guidelines for colour measurements were followed and spectrally

pure glass (CR-A51, Minolta Co., Osaka, Japan) was put between the samples and the equipment[2,5].

pH determinations were taken using a Crison 507 pHmeter and a Crison CAT. n° 52-32 electrode (Crison Instruments, S.A., Alella, Barcelona, Spain).

Statistical analysis: To analyse the effect of mincing on pH and lightness, a two-way ANOVA with interaction was carried out, considering the shoulder as a random factor (3 levels) and the treatment (whole lean meat and minced meat without additives, 2 levels) as a fixed factor.

To analyse the other treatments, a three-way ANOVA with all interactions was undertaken, considering the shoulder (3 levels) as a random factor and time (13 levels) and treatment (11 levels) as fixed factors.

To find where there were significant differences among the levels of the main factors or their interactions, contrasts between means were made. Tukey test, was applied when these contrasts were considered a priori, or Scheffe test, when these contrast were suggested by the experimental results[30,32].

To carry out the ANOVAs, the program 8V was used of the BMDP (version 9.0) statistical software.

RESULTS AND DISCUSSION

pH: Table 2 shows the ANOVA results of the pH for the main shoulder and treatment (mincing) factors and their interaction. It shows that the differences between pH, that the shoulders studied had, were significant ($p<0.01$), but that these differences were not present for the treatment (mincing).

Table 2: ANOVA results of pH for main Shoulder (S) and Treatment (mincing) (T) factors and their interaction (S x T)

Source	Sum of squares	DF	Mean square	F- ratio	Prob.
Shoulder (S)	2.5818	2	1.2909	29.28	0.0000
Treatment (T)	0.0000	1	0.0000	0.00	0.9927
S x T	0.0775	2	0.0388	0.88	0.4255
Error	1.3228	30	0.0441		

Prob.: Probability; DF: Degrees of Freedom

Tabla 3: ANOVA results of Llightness (L*) for main Sshoulder (S) and Treatment (T) (mincing) factors and their interaction (S x T)

Source	Sum of squares	DF	Mean square	F- ratio	Prob
Shoulder (S)	11.3386	2	5.6693	1.14	0.3269
Treatment (T)	93.9048	1	93.9048	23.11	0.0407
S x T	8.1272	2	4.0636	0.82	0.4464
Error	237.7866	48	4.9539		

DF: Degrees of Freedom; Prob. Probability

This indicates that shoulder meat comminution does not affect pH.

The mean pH values of the three shoulders were: 6.23 (shoulder n° 1), 6.74 (shoulder n° 2) and 6.13 (shoulder n° 3). When the pH contrasts were made (Scheffe test), significant differences ($p<0.01$) between shoulder n° 2 and shoulders n° 1 and n° 3 were found, but there were no significant differences between shoulders n° 1 and n° 3 ($p>0.05$).

Lightness (L*): The mean whole lean meat L* was 37.97±2.14) and that of the minced lean meat was 40.61±2.30. Table 3 shows the ANOVA results of the L* variable for the main shoulder and treatment (mincing) factors and their interaction. It was noted that significant differences ($p < 0.05$) existed as a result of mincing. This observation seems to indicate that, independently of the pH possessed by the shoulders themselves, on carrying out the mincing operation, the lightness of the lean meat increases.

In Table 4 the ANOVA results of the variable L* are shown for the main shoulder, time and treatments factors and their double and triple interactions. Firstly, it can be observed that the differences that existed for the lightness were significant ($p<0.01$) when the shoulder factor was considered. This means that the shoulders were different in their lightness. It may also be seen that L* did not present any significant differences ($p>0.05$) for the main time factor. Regarding the main Treatment factor (T), the table shows that there were significant differences ($p < 0.01$), which indicates that L* was modified by at least one of the treatments applied (Fig. 4).

When the contrasts were made (Tukey test), significant differences were found between the following treatments: minced lean meat (control) vs paprika, water

Table 4: ANOVA results of lightness (L*) for the main Shoulder (S), time (h) and Treatments (T) factors and their double and triple interactions

Source	Sum of square	DF	Mean square	F ratio	Prob.
Shoulder (S)	2.8536562E+3	2	1426.828	259.97	0.0000
Time (h)	8.8993324E+1	12	7.416	0.74	0.6970
Treatments (T)	2.5441625E+4	10	2544.163	16.39	0.0000
S x h	2.3912060E+2	24	9.963	1.82	0.0089
S x T	3.1041424E+3	20	155.207	28.28	0.0000
T x h	5.8049959E+2	120	4.837	1.03	0.4312
S x T x h	1.1326598E+3	240	4.719	0.86	0.9378
Error	1.8835951E+4	3432	5.488		

DF: Degrees of Freedom; Prob: Probability.
Treatments: Control, water, Salt (ST), Sodium Nitrite (SN), Sodium Ascorbate (SA), Lactic Acid (LA), Paprika (P), ST+SN, ST+SN+SA, ST+SN+P and LA+P

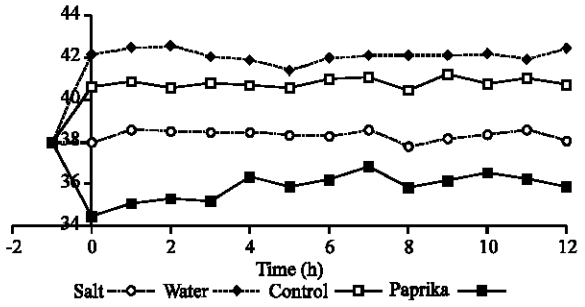

Fig. 1: Lightness (L*) evolution for minced lean meat (control) and water, salt and paprika treatments

vs paprika, salt vs lactic acid, sodium nitrite vs paprika, sodium ascorbate vs paprika, salt+sodium nitrite+sodium ascorbate vs salt+sodium nitrite+sodium ascorbate+ paprika ($p < 0.01$) and water vs salt+sodium nitrite and water vs salt ($p < 0.05$).

Figure 1 shows the evolution of L* for the following treatments: minced lean meat (control), water, salt and paprika. At time -1, the mean L* for the whole lean meat is shown and at time 0 the mean L* for the same lean meat after mincing is shown (representation of value -1 is only graphic). Taking the L* value at time -1 into account, it can be seen how lightness increases on mincing the lean meat, an observation that coincides with what has already been said. Moreover, that increase was greater when the lean meat was minced and 5% water was added. On the contrary, when the lean meat was minced and salt was added in addition to the 5% water, no increase in the L* was observed.

The extent to which meat appears glossy is related to the thin aqueous layer on the surface and the muscle's pH, water holding capacity, structure and fiber orientation[5].

Considering that the mincing took place under atmospheric pressure conditions and involves a process that destroys the tissue structure and incorporates air into the filling[3], it can be assumed, and be consistent with Palombo and Wijngaards[34], that the air included is one of the factors responsible for the increase in L*. However, it must not be overlooked that mincing leads to the partial liberation of the tissue liquids through the destruction of the structure itself. Those liquids will be found on the surface of the meat adding to the effect of the incorporated air. Bearing that in mind, it is probable that the increase in lightness due to mincing, is a result of the combined effect of the incorporated air, the liberation of tissue liquids and structural changes.

Table 5 shows the means (X) and the standard deviations (std) of L* for the minced lean meat (control) and each treatment.

As was previously mentioned, Table 4 shows that L* presented significant differences for the main treatment

Table 5: Means (X) and Standard Deviations (SD) of Lightness (L*) for the minced lean meat (control) and each treatment

Treatments	X	SD
Control	40.78	3.26
Water	42.10	2.96
Salt (NaCl)	38.30	1.88
Sodium Nitrite	41.30	2.86
Lactic acid	42.87	3.75
Sodium ascorbate	41.29	2.85
Paprika	35.82	2.40
Salt+nitrite	38.34	1.90
Salt+nitrite+ ascorbate	39.12	2.07
Paprika+lactic acid	37.53	2.71
Paprika+salt+nitrite+ascorbate	34.34	1.43

factor ($p<0.01$), but not for the time factor ($p > 0.05$). Considering that the contrast between the water and salt treatments indicated that the L* differences between both were significant ($p<0.05$) and that then there was no evolution in time, this shows that the effect of the salt on L* (Fig. 1 and Table 5), occurred at the moment of its being added.

Taking into consideration that the lean meat used in the experiment probably did not present its maximum Water Holding Capacity (WHC) owing to the pH (mean for three shoulders = 6.37), it seems logical to think that, for this reason, the 5% of water added to the mince could not be retained and formed a surface layer, so raising the L* value.

Salt alters the osmotic balance of the tissue[35]. When salt is added to the minced meat, it penetrates its structure, changes the electrostatic charges of the myofilaments[36,37] and affects the isoelectric point of the proteins, increasing its WHC[37-40].

The quantity of salt added during the experiment seems to have been sufficient to allow the 5% of water added to be retained by the meat's structure. This could explain how the salt compensated for the effect of the water and that of mincing on lightness.

Figure 2 shows the evolution of lightness differences ($\triangle$L*) over time for the following treatments: water in relation to the minced lean meat (control), salt in relation to water and the paprika treatment, using paprika powder, water and minced lean meat (control) as references. In this figure it can be seen how adding water makes the minced meat lighter (ÄL* +), while adding salt darkens it (ÄL* -).

There were no significant differences ($p > 0.05$) for L* between the water and lactic acid treatments, despite the decrease in pH due to the acid (mean meat pH in lactic acid treatments = 3.6 and 6.3 in water treatments) (Fig. 3). This indicates that the lightness is similar to that reached on adding 5% water. Taking the relationship of WHC and pH[38] into account, it can be assumed that, on adding the lactic acid, similar values of WHC will be reached as those obtained on adding 5% water. Nevertheless, it is known that when the pH is less than the isoelectric point of the

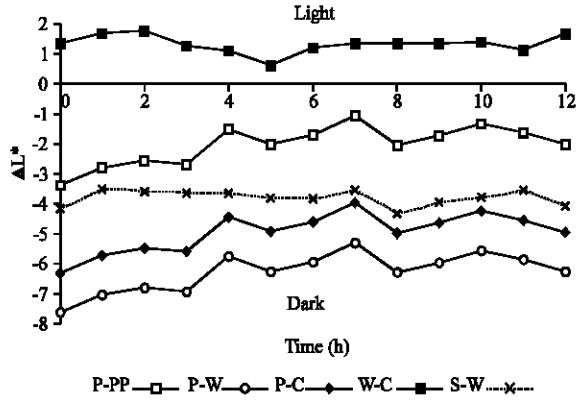

Fig. 2: Lightness differences (L*) for Paprika treatment using paprika powder (P-PP), water (P-W) and minced lean meat (control)(P-C) as references; water treatment using minced lean meat (W-C) as reference and salt treatment using water (S-W) as reference

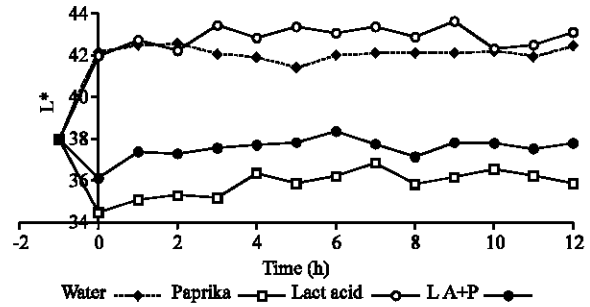

Fig. 3: Lightness (L*) evolution for water, paprika, lactic acid (Lact. acid) and Lactic Acid + Paprika (LA+P) treatments

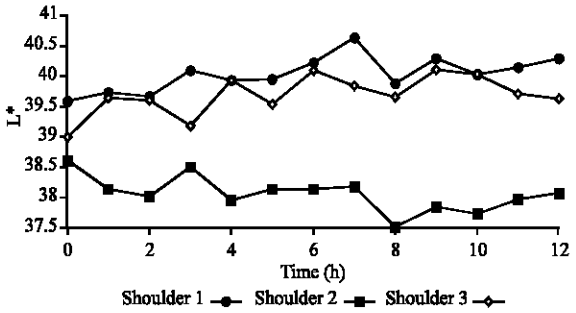

Fig. 4: Lightness (L*) Shoulder (S) x Time (h) interaction

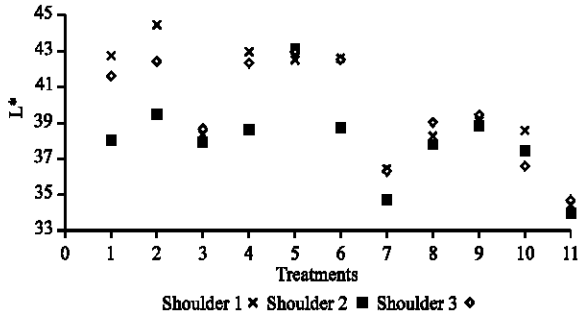

Fig. 5: Lightness (L*) Shoulder (S) x Treatments (T) interaction for minced lean meat (control) (1) and the treatments: water (2), salt (3), sodium nitrite (4), lactic acid (5), sodium ascorbate (6), paprika (7), salt+sodium nitrite (8), salt+sodium nitrite+sodium ascorbate (9), paprika+lactic acid (10) and salt+sodium nitrite+sodium ascorbate+paprika (11)

proteins (4.7 actin, 5.4 myosin[37], those proteins are denaturalized and alter their structure, thus modifying their light reflection and refraction properties[7,41], so the influence of the muscular fibre comminution must not be underestimated owing to the denaturalizing effect of the acid on the L*.

The contrast (Tukey test) between the salt and lactic acid treatments showed that the differences were significant ($p<0.01$). This behaviour is explained by what has already been observed for both, in terms of the salt and lactic acid treatments.

In Table 5 and Fig. 1, it can be seen that the mean L* value diminishes when paprika is added to the minced meat. The lightness reached with the paprika was less than the lightness found in the water and salt treatment. However, when the contrasts (Tukey test) were carried out, only the differences between the minced meat and the salt and paprika treatments were significant ($p < 0.01$).

In those contrasts (Tukey test) it was observed, that both the ascorbate and the nitrite had significant differences ($p<0.01$) with the paprika treatment. On the contrary, the ascorbate and the nitrite did not present significant differences ($p>0.05$) with water and minced meat. This would indicate that both the ascorbate and the nitrite do not affect the lightness of minced meat and that the L* value was in both cases a result of the paprika, which hydrated retaining 5% of the added water and did not allow it to remain free on the meat's surface. Similar behaviour was obtained by Fernández-López et al.[22] in red type dry-cured sausages.

In Fig. 3 the lightness behaviour for the water, lactic acid, paprika and paprika+lactic acid is shown over time. It was observed that L* values, when the lactic acid was added, were similar to those reached with the water (non-significant differences $p>0.05$, Tukey test). However, the values obtained both with the paprika and with the paprika+lactic acid, were less than the former ones

(significant differences $p<0.01$, Tukey test), but did not present any significant differences between themselves ($p>0.05$, Tukey test). This seems to indicate that the paprika, inhibits the lactic acid effect.

Paprika, like other spices absorbs water. This absorption depends on the high molecular weight content (starch, proteins) and on the size and number of particles[14]. In the case of paprika, the quantity of water retained can be 0.9 - 1.4 times its weight[42].

It is probable that the decrease in L*, the darker color of the lean pork and the inhibition of the lactic acid effect, are due to the paprika's capacity to retain water and the added acid.

In Fig. 2 it can be seen how water treatment lightens the meat, whilst the others darken it. Likewise, a greater darkening as a result of the paprika, was observed when the water was added to the minced meat, which may indicate that the paprika darkens on adding water.

The combined treatment with salt+sodium nitrite in contrast to the water treatment showed significant differences ($p<0.05$), which are a result of the individual effects of the salt and water.

In Table 4 it was observed that there were significant differences ($p<0.01$) for the shoulder-by-hours and shoulder-by-treatment interactions. The first of the interactions indicates that the shoulder presented different evolutions over time. When the contrasts for linear effects (Scheffe test) were made, there were no significant differences ($p>0.05$) between shoulders n° 1 and n° 3, but there were between those together two and shoulder n° 2 ($p<0.01$).

Water holding capacity is related to pH value[33]. Bearing in mind that shoulder n°2 had a higher average pH than the other two, it may be considered that it presented a greater water holding capacity and, therefore, its lightness is less.

The shoulder-by-treatment interaction indicates that there were different responses from the shoulder to the different treatments. In Fig. 5, the responses of the shoulders to the treatments under study are given.

It can be seen that both in the minced meat (control) and in the water, nitrite and ascorbate treatments, the shoulders showed similar behaviour. On carrying out the contrasts (Scheffe test) there were no significant differences ($p>0.05$) between the shoulders n° 1 and n° 3, but there were ($p<0.01$) between them and shoulder n° 2.

Also, it may be observed that in the salt treatments and their combinations (salt+nitrite, salt+nitrite+ ascorbate, salt+nitrite+ascorbate+paprika), the three shoulders behaved similarly. On carrying out the contrasts (Scheffe test) there were no significant differences ($p>0.05$) between the three shoulders. This seems to indicate that the salt eliminates the L* differences that initially existed between the shoulders.

Adding salt increases water holding capacity[38], so the L* of shoulders n° 1 and n° 3 diminished. Providing there is a direct relationship between WHC and L*, it is possible that the quantity of salt added originated a maximum WHC coinciding with that which shoulder n° 2 initially had owing to its pH. Nevertheless, the possibility that there may be a WHC value above or below which lightness depends on other factors, cannot be discarded.

In Fig. 5, moreover, it can be observed that, in the treatments with paprika (n°7 and 11), L* diminished below the values of similar treatments without paprika (n°1 and 9). On carrying out the contrasts (Scheffe test) no significant differences were observed ($p>0.05$) between the three shoulders of treatments n°7 and n°11. On the contrary, significant differences ($p<0.01$) were observed between the treatments with paprika (n°7, 10 and 11) and similar ones without paprika (n°1, 5 and 9).

On treating the minced meat with lactic acid (Fig. 5, treatment 5) it was observed that L* increased and that the initial differences between the shoulders ($p > 0.05$ Scheffe test) disappeared. This behaviour shows that the structural disorganization caused by the lactic acid was a determinant for the L* value.

In treatment n°10 (Fig. 5) it can be seen that combined adding of lactic acid and paprika to the minced meat produced a decrease in L* in relation to the treatment with lactic acid (treatment n° 5) (significant differences $P < 0.01$, Scheffe test). The differences between the three shoulders in treatment n°10 were non-significant ($P > 0.05$). Despite the lightness differences initially observed between the shoulders (control), the denaturalizing effect of the lactic acid may cause such a structural disorganization as to eliminate those differences.

CONCLUSIONS

Mincing produces an increase in the lean meat's lightness at the moment of carrying out the treatment, which does not evolve for the following 12 h. Adding 5% water to the minced meat does not result in any significant changes to its lightness, but increases lightness in relation to whole lean meat. The salt treatment (2.3%) compensates for the effect that the mincing and adding of water has on the meat's lightness. This takes effect at the moment of adding salt. The salt added to minced pork of different pHs makes their L* values uniform.

The treatments using sodium nitrite (0.01%) and sodium ascorbate (0.05%) do not change the L*.

Adding paprika darkens the minced meat, an effect which takes place at the moment of its being added and which does not evolve over time. The salt, lactic acid and paprika make L* uniform, in minced lean meat with different pHs.

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