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Physicochemical characteristics of Spanish-type dry-cured sausage

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Abstract

The colour and physicochemical parameters of Spanish-type dry-cured sausage during fermentation and ripening were evaluated. The CIE L^*, a^*, b^* and pH, moisture content, lactic acid concentration and residual nitrite level were measured in two different zones (centre and peryphery). Sausage processing had two stages, fermentation and ripening. Lightness (L^*) was the only colour parameter whose development (during the ripening stage) was dependent on the zone studied, in this case becoming lighter in the center zone. Redness (a^*) increased during the fermentation stage and decreased during ripening, while the yellowness (b^*) decreased throughout the process. The development of pH, lactic acid and residual nitrite levels were non related to the zone studied during both the fermentation and ripening stage.

Keywords: CIELAB colour space; Dry-cured sausage; pH, ripening; Fermentation; Lactic acid; Moisture; pH

1. Introduction

Dry-cured sausages are one of the oldest forms of preserving meat and are typical of Mediterranean countries with a dry climate (Spain, Italy, France, Portugal and Turkey). In contrast, smoke-cured sausages, or cooked sausages prevail in countries with a colder climate (Bowers, 1991).

The name of some Spanish dry-cured sausages comes from the latin term "salsus" which means meat preserved by salting. The process involves a casing with minced or roughly cut meat and fat, minced or roughly cut along with salt and spices. In Spain, dry-cured sausages can be divided into two types: fermented sausages ("chorizo", "salchichón" and "fuet") and non-fermented sausages ("longaniza de pascua") (MAPA, 1983), although, all these sausages tend to be classified as fermented (Leistner, 1995).

At the moment, fermented sausages, even though they are made with different recipes and use different technologies, have the common properties of being formulated with lean meat and fat (at different proportions depending of the recipe), being able to be stored at room temperature and having a high microbial load (Baldini, Farina & Palmia, 1981).

Spain is the main consumer country in terms of dry-cured sausages (3.2 kg, per inhabitant/year), of which "chorizo" (61,700 t) and "salchichón" (61,000 t) are the most popular (Mill, Mahlau & Furitsch, 1996).

Spanish dry-cured sausages are contained in many different diameter casings, but never less than 20 mm. Industrially, most sausages are made in casings with 55 mm, which differentiates them from traditional products.

In Spanish type sausage, the dry-cured process can be divided into two main stages: fermentation and ripening. During fermentation, a slow but substantial heating takes place, which is very important for encouraging the growth of the lactic acid bacteria producers (Acton & Dick, 1977). These micro-organisms, together with the lipolytic and proteolytic enzymes (Roncalés, 1994) determine the characteristics of the final product. At the end of the ripening stage, an intermediate moisture food is obtained.

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The physical, chemical and biochemical changes that take place during processing have not been adequately studied and very few papers study colour evolution in this type of meat product (Pagán-Moreno et al., 1997). Several authors have reported that moisture loss during manufacture of some meat products could affect the colour properties (Perlo, 1997; Sayas, 1997). Pérez-Alvarez (1996) reported that surface hardening in the dry-cured meat products also affects the colour parameters.

The objective of this work was to study the development of the colour (CIELAB colour space) and chemical properties of Spanish-type dry-cured sausage during processing and to compare these characteristics in different zones (centre and periphery) of the sausage.

2. Materials and methods

2.1. Processing and sampling

Three batches, each batch consisting of 50 sausages (500 g each sausage), were processed in a pilot plant. The following ingredients were used: lean pork meat (shoulder) 66.20%, pork fat (backfat) 28.22%, sodium chloride 2.50%, lactose 2.0%, dextrose 1.0%, sodium ascorbate 0.05%, potassium nitrate 0.02% and sodium nitrite 0.01%.

Sausage processing lasted a total of 24 days, starting from when the sausage casing was filled. The Spanish-type dry-cured sausage process consisted of the following steps:

a. Mincing: the meat (lean pork) and fat (pork backfat), was chopped and, introduced into a mincing machine (CATO 114, Sabadell, Spain) with a plate of 8mm diameter holes (Olotinox, Olox, Spain).
b. Mixing: the minced meat and the fat, were passed through a mixer (CATO "de brazos", Sabadell, Spain), in which they were mixed with the other ingredients that had previously been dissolved in water (5%). The mixing time to obtain the Spanish-type dry-cured sausage filling was 3–5 min.
c. Resting: the filling was left to rest for 24 h in cold storage at $2 \pm 1°C$.
d. Stuffing: The filling was stuffed into FIBRAN artificial casings (55 mm diameter) (FIBRAN, Pilarica, Spain) using a piston stuffer (CATO, Sabadell, Spain).
e. Draining: the sausages were drained in a cold store ($2 \pm 1°C$) for 5 h.
f. Fermentation: The sausages were kept for 48 h in a room with controlled relative humidity ($92 \pm 2\%$), temperature ($22 \pm 2°C$) and air velocity (0.20 ± 0.05 m/s). For this stage no starter culture was used.
g. Ripening: finally the "sausages" were dried over a period of 22 days under the following conditions: temperature $14 \pm 2°C$, relative humidity $88 \pm 2\%$, and air velocity 0.20 ± 0.05 m/s.

After the draining stage, three sausages of each batch were analysed at the following times: 0, 12, 24, 36, 48 h (fermentation stage) and 6, 12, 18 and 24 days (ripening stage).

Three portions of each sausage were cut along their cross-sectional diameter. Two areas were considered in each of the cut portions: the "centre" (10 mm radius from the centre) and the periphery (from the central zone to the casing). Readings were carried out in triplicate on each zone of each portion (a total of 1350 observations were made for each variable).

2.2. Chemical analysis

2.2.1. Moisture content

Moisture content (%w/w) was determined in accordance with ISO standard 1442 ISO, (1975a) and the results were expressed as water (g)/100 g tissue.

2.2.2. Residual nitrite level

Residual nitrite level was determined in accordance with standards ISO/DIS 2918 (ISO, 1975b). Absorbance readings were taken using an HP 8451 Array Diode spectrophotometer and results were expressed as parts per million (ppm).

2.2.3. Lactic acid content

The lactic acid content was determined in accordance with standard AOAC 1504 (1990) and results were expressed as %w/w lactic acid.

2.2.4. pH

The pH determinations were taken using a Crison 507 pHmeter and a Crison CAT. 52-32 electrode (Crison Instrumets, S.A., Alella, Barcelona, Spain.) in accordance with Spanish Ministry of Health regulations (Ministerio de Sanidad y Consumo, 1985).

2.3. Colour analysis

Colour measurements were taken immediately after cutting the samples (so as to prevent colour degradation as a result of light and oxygen) in accordance with the recommendations on colour determination and pH of the American Meat Science Association (Hunt et al., 1991).

The CIELAB colour space was studied in accordance with Cassens et al. (1995). The following colour coordinates were determined: lightness (L^*), redness (a^*, ± red–green) and yellowness (b^*, ± yellow–blue).

The colour parameters were determined using a Minolta CM300 colorimeter (Minolta Camera Co., Osaka, Japan). Illuminant D_{65} was chosen and a 10° standard observer (Cassens et al., 1995). American Meat Science Association Guidelines for colour measurements were followed (Hunt et al., 1991) and spectrally pure glass

(CR-A51 Minolta Camera Co., Osaka, Japan) was put between the samples and the equipment.

2.4. Statistical Analysis

Conventional statistical methods were used to calculate means and standard deviations. Statistical analysis (ANOVA) was applied to the data to determine stastically significant differences ($P \leqslant 0.05$) between each factor. To discover if there were significant differences between the levels of the main factors, Tukey's test was applied between means (Afifi & Azen, 1979). Two ANOVAs with two factors (time and zone) were applied for each parameter, one for the fermentation stage (time: 0, 12, 24, 36 and 48 h, and zone: centre and periphery) and the other for the ripening stage (time: 6, 12, 18 and 24 days, and zone: centre and periphery). The statistical data analysis was undertaken using the statistical package BMDP version 9.0 (BMDP, 1993).

3. Results and discussion

3.1. pH and lactic acid

ANOVA results for pH and lactic acid indicated significant differences ($P \leqslant 0.05$) between times during fermentation and during ripening, but not between zones (Tables 1 and 2). Fig. 1 shows pH and lactic acid values during the fermentation and ripening stages. To know at which times the levels were significantly different, contrasts were made using the Tukey test and significant differences ($P \leqslant 0.05$) between all the times were detected for both the fermentation and ripening stage. The decrease in pH that was observed during fermentation stage may be due to microbial activity, since the microorganisms metabolize the sugars (dextrose and lactose) present in the meat batter into lactic acid (Flores & Alvarruiz, 1985). In this stage, ideal microorganism growth conditions are established. At the end of the fermentation stage (48 h) a pH of 5.1 ± 0.1 was obtained. This pH was less than the isoelectric point of myosin (Wismer–Pedersen, 1994).

The increase in pH during the ripening stage that can be observed in Fig. 1 is similar to that reported for other Spanish dry-cured meat products such as "lomo embuchado" (Pérez-Alvarez, 1996), "dry-cured ham" (Sayas, 1997), "chorizo", (Pagán-Moreno et al., 1997), "longaniza de pascua" (Pérez-Alvarez, 1996) and "fuet" (Roncalés, 1994). Roncalés reported that this increase was due to mold growth. The type of sausage studied by us showed a white mold on the surface during the ripening stage (12–24 days).

The behaviour of lactic acid in dry-cured sausages helps to evaluate the microbial flora activity of this

Table 1
ANOVA results for colour coordinates [lightness (L^*), redness (a^*) and yellowness (b^*)] and physicochemical parameters (pH, residual nitate level, moisture content and lactic acid concentration) of Spanish-type dry-cured sausage during fermentation stage

Variable	Source	Sum of squares	Df	Mean square	F-ratio	P-value
L^*	Time (T)	543.50166	4	135.87542	5.93	0.0040
	Zone (Z)	7.70680	1	7.70680	0.37	0.5488
	Interaction (TZ)	70.37053	4	17.59263	0.68	0.6173
	Residual	500.01895	16	20.83412		
a^*	Time (T)	543.43921	4	135.85733	5.93	0.0040
	Zone (Z)	4051.03962	1	4051.03962	3.12	0.0963
	Interaction (TZ)	137.35472	4	27.47094	0.44	0.777
	Residual	309.73423	16	12.90559		
b^*	Time (T)	352.09213	4	88.02303	5.57	0.0053
	Zone (Z)	533.95378	1	533.95378	4.19	0.0568
	Interaction (TZ)	181.24753	4	45.31188	0.44	0.7823
	Residual	252.86298	16	15.80394		
Residual	Time (T)	1093.14515	4	273.28629	483.23	0.0000
nitrate level	Zone (Z)	0.20160	1	0.20160	1.71	0.2039
	Interaction (TZ)	64.17445	4	16.04361	2.21	0.1139
	Residual	0.85785	16	0.03574		
pH	Time (T)	2.23097	4	0.55774	86.83	0.0000
	Zone (Z)	0.09587	1	0.09587	0.49	0.4932
	Interaction (TZ)	1.47819	4	0.36955	1.54	0.2392
	Residual	0.10278	16	0.00642		
Lactic acid	Time (T)	0.11027	4	0.02757	11.78	0.0001
	Zone (Z)	0.29843	1	0.29843	1.24	0.2819
	Interaction (TZ)	1.12218	4	0.28055	1.44	0.2666
	Residual	0.03746	16	0.00234		
Moisture	Time (T)	7504.10657	4	1876.0266	96.04	0.0000
	Zone (Z)	0.08591	1	0.08591	0.00	0.9787
	Interaction (TZ)	110.56990	4	27.64248	0.24	0.9130
	Residual	1862.14770	16	116.38423		

Table 2
ANOVA results for colour coordinates [lightness (L^*), redness (a^*) and yellowness (b^*)] and physicochemical parameters (pH, residual nitrite level, moisture content and lactic acid concentration) of Spanish-type dry-cured suasage during ripening stage

Variable	Source	Sum of squares	Df	Mean square	*F*-ratio	*P*-value
L^*	Time (T)	684.075	3	228.025	124.86	0.0000
	Zone (Z)	25.4077	1	25.4077	13.91	0.004
	Interaction (TZ)	421.092	3	140.364	2.72	0.0517
	Residual	116.883	64	1.8263		
a^*	Time (T)	226.252	3	75.4172	29.23	0.000
	Zone (Z)	2.45681	1	2.45681	0.95	0.3328
	Interaction (TZ)	7.60264	3	2.53421	0.98	0.4068
	Residual	165.122	64	2.58003		
b^*	Time (T)	595.015	3	198.338	109.59	0.000
	Zone (Z)	13.6068	1	13.6068	1.69	0.1988
	Interaction (TZ)	28.986	3	9.66199	1.20	0.3180
	Residual	115.827	64	1.809979		
Residual	Time (T)	2046.84	3	682.278	0.77	0.5093
nitrate level	Zone (Z)	1362.07	1	1362.07	1.55	0.2149
	Interaction (TZ)	2612.91	3	870.971	0.99	0.3988
	Residual	246829.0	64	881.455		
pH	Time (T)	35.0362	3	11.6787	4.50	0.0063
	Zone (Z)	0.190139	1	0.190139	0.07	0.7875
	Interaction (TZ)	18.7524	3	6.25081	0.94	0.4275
	Residual	166.105	64	2.5954		
Lactic acid	Time (T)	153.991	3	51.3304	6.74	0.0005
	Zone (Z)	5.13067	1	5.13067	0.67	0.4147
	Interaction (TZ)	38.0946	3	12.6982	1.67	0.1827
	Residual	487.237	64	7.61308		
Moisture	Time (T)	1489.13	3	840.261	26.05	0.0000
	Zone (Z)	840.261	1	496.377	44.11	0.0000
	Interaction (TZ)	41.9243	3	13.9748	0.73	0.5328
	Residual	5334.36	64	19.0513		

product, which is mostly due to lactic acid bacteria and, secondly, to *Micrococcaceae*. In the fermentation stage, the room conditions (temperature and relative humidity) favour the exponential growth stage of the micro-organisms, whilst in the ripening stage, the micro-organisms are in a dormant phase (Banwart, 1982).

Fig. 1 shows the evolution of lactic acid during the elaboration process. There is a much stronger increase in lactic acid during the fermentation stage (48 h) than in the ripening period (note the change in the steepness of the curves between both phases). This lower increase in lactic acid concentration during the ripening stage can be attributed to the fact that after exhausting the easily fermentable sugars, the microorganisms are now using the slow fermenting sugars (lactose) (Zert, 1980).

The pH is closely linked to the development of the % of lactic acid ($R^2 = -0.887$; $P \leqslant 0.05$).

3.2. Moisture

ANOVA results for moisture show that significant differences existed between times and zones ($P \leqslant 0.05$) during the ripening stage (Table 2), but only between times during the fermentation stage ($P \leqslant 0.05$) (Table 1). Fig. 2 shows moisture levels during the fermentation and ripening stages for the two zones under study.

The Tukey test applied to the time factor for the fermentation stage showed that there were no significant differences ($P > 0.05$) between 0 and 12 h, or between 24 and 36 h, but the differences were significant ($P \leqslant 0.05$) between all these times and 48 h. During this stage moisture decreased (Fig. 2). The room conditions, particularly the high relative humidity during the fermentation stage, presented excessive moisture lost and encouraged microorganism growth.

The Tukey test applied to the time and zone factors in the ripening stage showed that significant differences ($P \leqslant 0.05$) existed between each time and zone during this stage, the moisture of the sausage diminished, particularly in the periphery zone.

Development of this parameter allows one to follow the drying process, during which the sausage's free surface water evaporates while the free internal water diffuses towards the periphery. Various mechanisms are responsible for this displacement of water in the sausage: the diffusion of liquid water due to concentration gradients, the movement of liquid water by capillary force (which is also proportional to the water concentration gradient of the product), diffusion of layers of liquid water adsorbed on interphases, the movement of liquid water caused by the contraction of the product during the drying stage, diffusion of water vapour as a

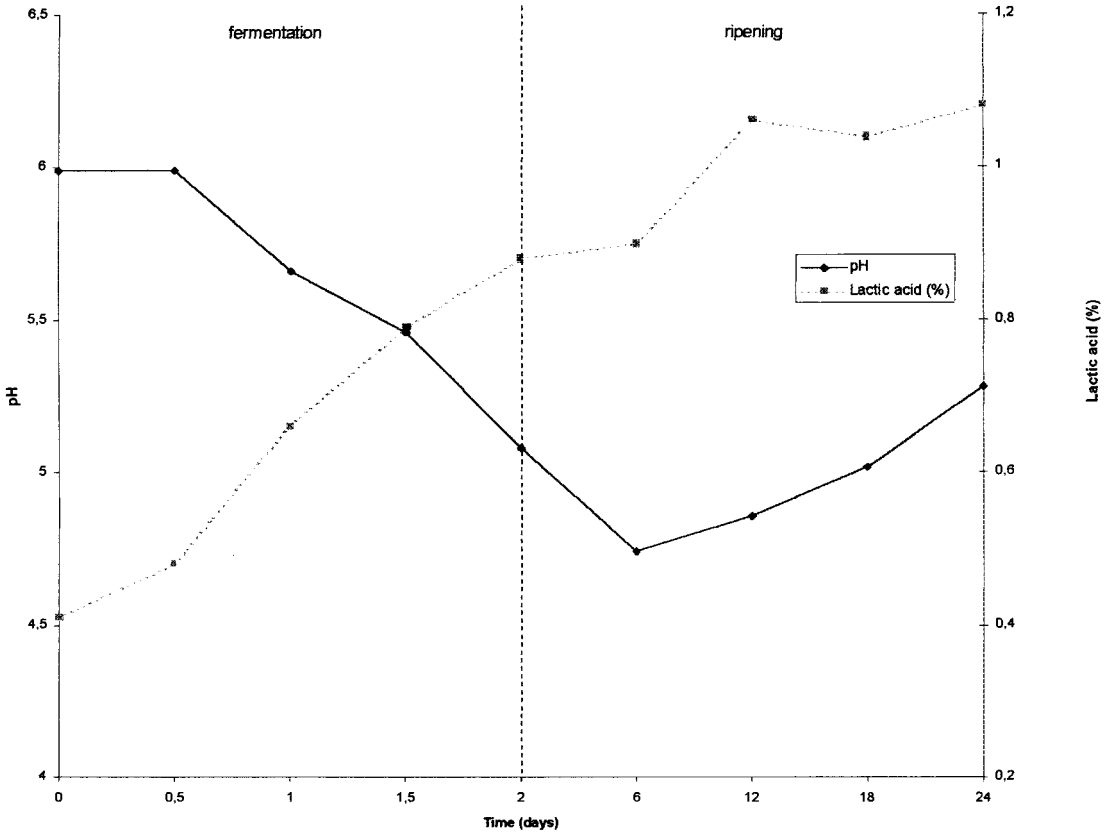

Fig. 1. pH and lactic acid (%) during the fermentation and ripening stages in a Spanish-type dry-cured sausage (81 determinations have been used to generate each data point).

result of partial pressure gradients, movement through successive evaporation-condensation processes and movement through successive desorption-adsorption processes (Pérez-Alvarez, 1996).

3.3. Residual nitrite level

ANOVA results for residual nitrite levels only showed significant differences between times ($P \leqslant 0.05$) for the fermentation stage (Table 1), but not for the ripening stage ($P > 0.05$) (Table 2). Fig. 3 shows residual nitrite levels during the fermentation and ripening stages.

The Tukey test applied to the time factor for the fermentation stage showed that the differences between 36 and 48 h, were not significant ($P > 0.05$) but they were significantly different ($P \leqslant 0.05$) between these times and the other times (0, 12 and 24 h). During this stage residual nitrite levels decreased (Fig. 3).

In the fermentation stage, the differences over time could be attributed to the fact that the concentration of residual nitrite diminishes during the dry-curing process, due to several mechanisms: transformation into other compounds (nitrates), reaction to other meat components (proteins, cytochromes, etc.) and the presence of ascorbate acid and its salts (Perlo, 1997). In the ripening stage significant differences were not found for the time factor (Table 2) because the nitrite had reacted with the meat compounds in the previous stage. This behaviour has been observed in other dry-cured meat products such as "chorizo" (Pagán-Moreno et al., 1997) and "dry-cured ham" (Sayas, 1997).

The fact that there were no significant differences between zones may be due to the fact that the curing agents are added in water (for good dispersion) at the mixing stage, which means that, the curing salts are distributed in the most homogeneous way possible from the outset.

3.4. Lightness (L*)

ANOVA results for lightness indicated significant differences due to time ($P \leqslant 0.05$) during the fermentation stage (Table 1), but during the ripening stage, significant differences ($P \leqslant 0.05$) were attributed to time and zone (Table 2). Fig. 4 shows changes in the parameter lightness during the fermentation and ripening stage for the two zones under study.

The Tukey test applied to the time factor for the fermentation stage showed that there were no significant differences ($P > 0.05$) between 0 and 48 h, nor between, 12, 24 and 36 h, although there were significant differences ($P \leqslant 0.05$) between these two time groups. The decrease in L^* during the first 36 h (Fig. 4) could be

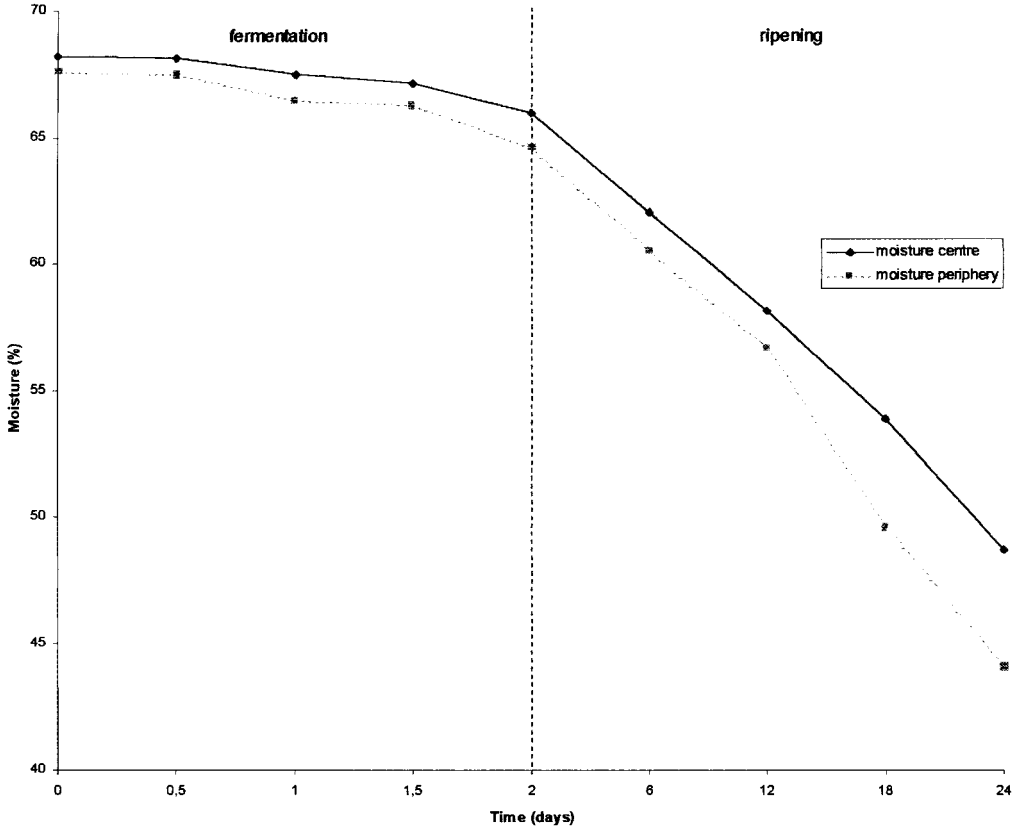

Fig. 2. Moisture content (%) during the fermentation and ripening stages in different zones (centre and periphery) in a Spanish-type dry-cured sausage (81 determinations have been used to generate each data point).

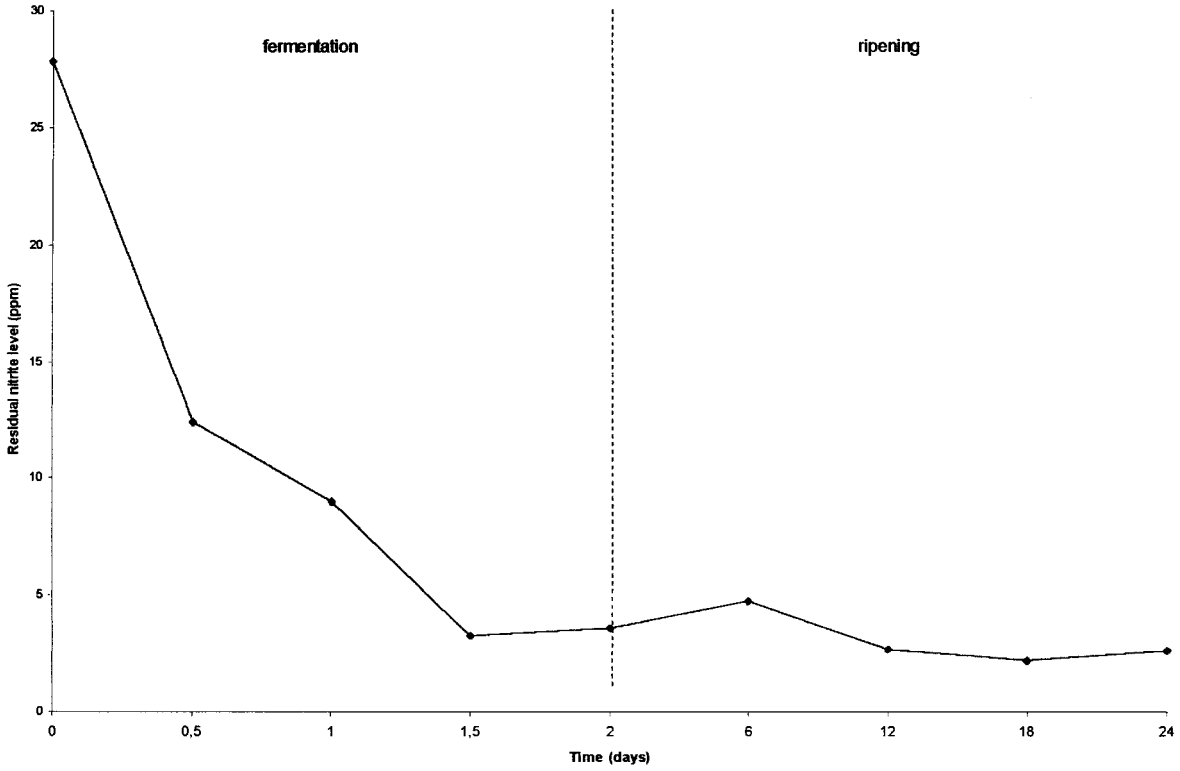

Fig. 3. Residual nitrite level (ppm) during the fermentation and ripening stages in a Spanish-type dry-cured sausage (81 determinations have been used to generate each data point).

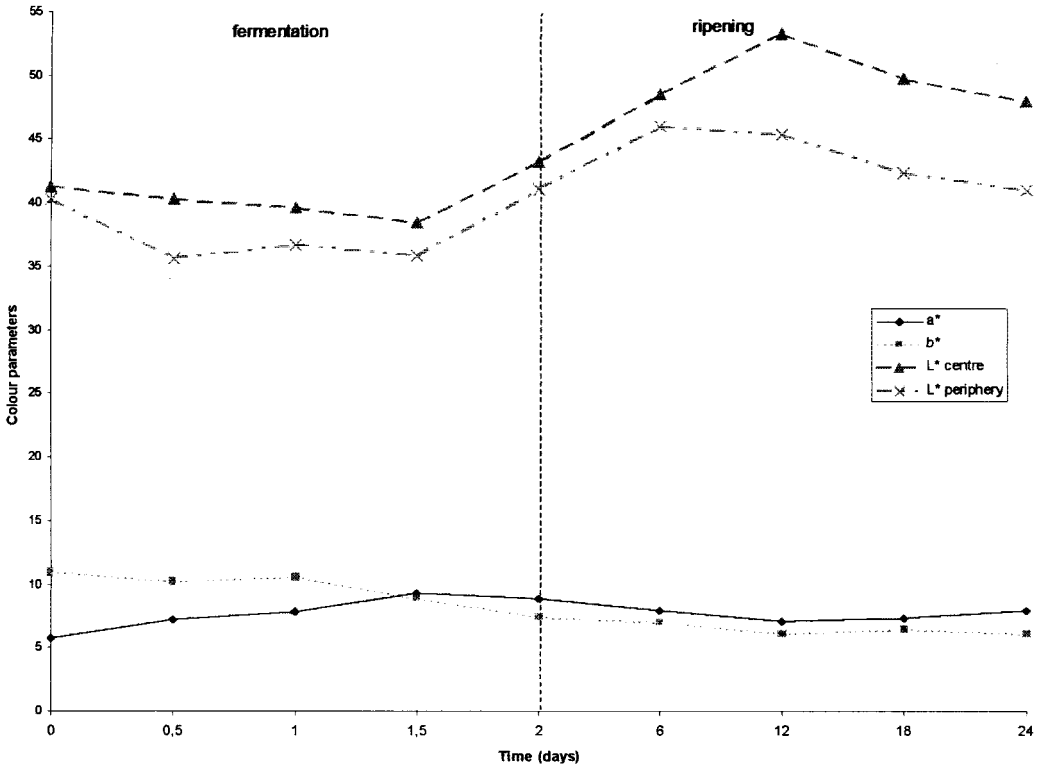

Fig. 4. Lightness (L^*), redness (a^*) and yellowness (b^*) during the fermentation and ripening stage in a Spanish-type dry-cured sausage (81 determinations have been used to generate each data point).

attributed to moisture lost, such as occurs, in other dry-cured (Pérez-Alvarez, 1996; Sayas, 1997) and cooked meat products (Perlo, 1997). The increase observed after 36 h and during the first 12 days of the ripening stage could be attributed to the changes in lactic acid and pH that cause exudation in the meat because the meat proteins have reached their isoelectric point. This was consistent with observations in the salting-seasoning stage of "lomo embuchado" (Pérez-Alvarez et al., 1997).

The Tukey test applied to time and zone factors in the ripening stage showed significant differences ($P \leqslant 0.05$) between each time and zone under study. With regards to the significant differences found between zones (lower values in the peripheral zone than in the centre) (Fig. 4), these may be attributable to the moisture gradient in the sausage and the differences in relative humidity between the drying-room atmosphere and the sausages (Rosmini, 1997). The same effect has been observed in different zones of "lomo embuchado" (Pérez-Alvarez et al., 1997).

3.5. *Redness* (a*)

ANOVA results for redness showed that significant differences existed between times ($P \leqslant 0.05$) in both the fermentation and ripening stage but not between zones ($P > 0.05$) (Tables 1 and 2). Fig. 4 shows redness development during fermentation and ripening.

The Tukey test applied to the time factor in both the fermentation and ripening stage showed significant differences ($P \leqslant 0.05$) between each time. During the fermentation stage redness increased but diminished during the ripening stage (Fig. 4). The increase in redness during the fermentation stage could be attributed to the formation of nitrosomyoglobin which has been previously related with the characteristic red colour of this type of meat product (Bruggen, 1994). Fernández-López (1998) reported that the salt content was responsible for increases on this colour co-ordinate in a dry-cured sausage model system. The increase in a^* observed during fermentation may have also been due to moisture loss, which would increase the salt content (on a wet basis). The decrease in a^* observed during the ripening stage (Fig. 4) can only be attributed to the effect of lactic acid effect on the different states of myoglobin (myoglobin, nitrosomyoglobin and oxymyoglobin). This acid might partially or totally denature this haemo-compound. Some authors have reported that this acid decreases redness (Fernández-López, 1998; García-Marcos, Rosmini, Pérez-Alvarez, Gago, López-Santoveña & Aranda, 1996).

With regard to the effect of salt on this co-ordinate, one might expect significant differences between zones since the periphery has a lower moisture and so higher salt content (on a wet basis) than the centre. This effect could promote higher redness in the periphery zone.

However, it has been reported that in a dry-cured sausage model system, the lactic acid effect is stronger than the effect of salt in mixtures of these two additives (Fernández-López, 1998). This dominance of lactic acid could be responsible for the effect that significant differences between zones were not found for redness.

3.6. Yellowness (b*)

ANOVA results for yellowness showed significant differences between times existed for the fermentation ($P \leqslant 0.05$) and ripening stages ($P \leqslant 0.05$), but not between zones ($P > 0.05$) (Tables 1 and 2). Fig. 4 shows yellowness during the fermentation and ripening stages.

The Tukey test applied to the time factor during the fermentation stage showed significant differences ($P \leqslant 0.05$) between all the times. Of note is the clear decrease that takes place during the fermentation stage (Fig. 4). The observed changes in b^* during fermentation are probably due to the oxygen consumption by microorganisms during their exponential growth phase and so the decrease in oxymyoglobin which greatly contributes to the value of this colour co-ordinate (Fernández-López, 1998; Johansson, Tornberg & Lundström, 1991). Other authors also mention that the micro-organisms produce metabolites that induce the oxidation of meat and fat present in the sausage (Demeyer, Verplaetse & Gistelinck, 1986; Sarasibar, Sánchez & Bello, 1989; Zert, 1980) and, by so doing, contribute to the decrease of this value. Fernández-López (1998) reported that the salt content lowers the value of b^* (due to the effect of salt on oxygen solubility in the meat batter), while the lactic acid concentration increases this colour co-ordinate in a dry-cured sausage model system. In accordance with these combined effect of these two additives, the decrease in yellowness observed during the fermentation stage could be due to the salt which dominates the effect of lactic acid, the concentration of which is low in this stage.

The Tukey test applied to the time factor in the ripening stage showed no significant differences ($P > 0.05$) between 12, 18 and 24 days although the difference between these times and 6 days were significant. In this stage the changes in this co-ordinate were less than in the fermentation stage (Fig. 4). In both stages the nitrite could be reacting with the myoglobin forming nitrosomyoglobin, and so the myoglobin and/or oxymyoglobin present might decrease and, with it, the b^* value. This behaviour has also been observed in other dry-cured meat products (Pérez-Alvarez, 1996; Pérez-Alvarez et al., 1997). In the ripening stage, the high lactic acid concentration reached might have been expected to increase this co-ordinate, although in fact a slight decrease was observed. This behaviour could indicate that the salt effect predominates upon lactic acid, in agreement with that reported by Fernández-López (1998) in meat batters for different concentrations of these additives. These results show that yellowness behaves in the opposite way to the redness co-ordinate.

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