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Study of the biochemical changes during the processing of Androlla, a Spanish dry-cured pork sausage

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Abstract

Androlla is a dry-cured sausage manufactured with pork in the northwest of Spain (Galicia). In three batches of this product, manufactured by three different producers, samples of the mix before stuffing and from sausages after 1, 3, 7, 14, 21, 28 and 42 days of ripening were taken. In each sample, the overall chemical composition, physico-chemical parameters, nitrogen fractions and some degradative parameters of the fat were determined. The changes in the different compositional parameters throughout ripening were similar to those observed in other similar products and the values of these parameters, at the end of ripening, were inside the wide range of values reported in the bibliography for various dry-cured sausages. Sugars were not added in the mix and the lactic fermentation during ripening was not very intense. The values of non-protein nitrogen, α-aminoacidic nitrogen and total basic volatile nitrogen were increased significantly during ripening. The final levels of non-protein nitrogen were found to be within the range of values observed for other sausage varieties; however, the values of α-aminoacidic nitrogen and total basic volatile nitrogen were lower than those found in similar products. From the values of the acidity of the fat it can be concluded that the lipolysis that this product undergoes is moderate; the peroxide values are higher than those found in other sausages, however T.B.A. values are lower to those habitually described by other authors.

Keywords: Androlla; Proteolysis; Lipolysis; Ripening; Dry-cured sausages

1. Introduction

Meat and meat products represent an important part of the human diet.

The origin of the manufacture of dry-cured meat sausages probably goes back to Babylonian times, when methods such as the drying, salting or fermentation of meat were already in use. Nowadays, we recognize dry-cured meat sausages as products with low water activity values, a suitable pH, characteristic aroma, typical red colour, consistency, cohesion when cut and a usefully long life without refrigeration.

The technology of dry-cured sausages allows many variations as long as the basic concepts (reduction of pH and water activity) are kept in mind. Consequently a great variety of these products exist in all the producer countries.

During the manufacture of these meat products, apart from microbiological changes, other chemical and physico-chemical modifications occur, especially dehydration, fermentation of carbohydrates and acidification, development of colour, lipolysis and autooxidation of lipids and proteolysis. These changes are responsible for the organoleptic characteristics of the final products.

Androlla is a traditional sausage manufactured in the northwest region of Spain (Galicia) and is included in the 'Católogo de Embutidos y Jamones curados de España' (MAPA, 1983). It is highly regarded among consumers in local markets. However, this traditional product does not achieve the uniform and consistent quality that the modern market demands, which limits its potential for new markets. This problem seems to be related to the fact that the changes that the constituents undergo throughout the ripening process are not fully understood. These changes contribute to the typical organoleptic characteristics of the product.

The studies carried out on this product until now only refer to the biochemical characteristics of the final product (Lorenzo, Michinel, López, & Carballo, 2000), and there is a lack of information about the chemical and

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physico chemical changes that occur throughout its manufacture.

The aim of this research was to study the changes of the contents of the chemical components (moisture, protein, fat, ash, NaCl, total carbohydrates and nitrate), the changes of the physico chemical parameters (water activity, pH and titratable acidity), and the degree of proteolysis and lipolysis during the processing of Androlla.

2. Materials and methods

2.1. Samples

In this study, Androlla samples were industrially produced in three different factories using traditional technologies. The production methods were the same in the three factories. The ingredients used in the preparation of the mix are summarized in Table 1; sugars and LAB starter cultures are not added. Meat was ground, ribs were cut into pieces of approximately 3 cm length, and the ingredients were minced for 15 min. The resulting mix was left standing for 12 h at 20 °C and was then stuffed into natural casing of 6.5 cm in diameter in units of 15–20 cm length. Subsequent ripening was carried out for 42 days in storerooms at 12 °C and 70% relative humidity.

From each factory, samples at 0 days (mix before stuffing), and after 1, 3, 7, 14, 28 and 42 days of ripening were taken. Each sample consisted of one entire Androlla sausage. Samples were taken to the laboratory under refrigeration (4 °C). In order to prepare the samples for analysis, in each Androlla sausage after removing and discarding the outer casing and the bones, the edible part was thoroughly cut up into small pieces and ground in a Moulinette (Moulinex/Swan Holdings Ltd., Birmingham, England) mincer until a homogeneous mass was obtained. After determining the moisture content, water activity and pH, samples were stored frozen in airtight bottles at-80 °C for no longer than 4 weeks prior to further analysis.

Table 1
Composition of the mix used in the manufacture of the Androlla batches

Ingredients	kg/100 kg
Ribs with their fleshy parts	66
Pork jowl	4.7
Lean pork	18.8
Pork back fat	4.7
Salt	2.8
Sweet paprika	2.4
Spicy paprika	0.4
Garlic	0.1
Marjoram	0.1

2.2. Analytical methods

Moisture, fat, protein (Kjeldahl N×6.25), ash, NaCl, and nitrate contents were quantified according to the ISO recommended standards 1442:1997 (ISO, 1997), 1443:1973 (ISO, 1973), 937:1978 (ISO, 1978), 936:1998 (ISO, 1998), 1841–1:1996 (ISO, 1996), and 3091:1975 (ISO, 1975), respectively.

Total carbohydrates were quantified using the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, Smith, 1956) in an extract obtained with 0.6 N $HClO_4$, following the procedure of De Ketelaere, Demeyer, Vandekerckhove, and Vervaeke (1974). This same extract was used in the determination of the total non-protein nitrogen (NPN) using the Johnson (1941) method, the α-aminoacidic nitrogen (NH_2-N) using the Moore and Stein (1948) method, and the total basic volatile nitrogen (BVN) using the Pearson (1968) method.

pH was measured with a pH meter micro pH 2002 (CRISON Instruments, S.A., Barcelona, Spain) after mixing 10 g of sample with 10 ml of distilled water for 2 min. in a Sorvall Omnimixer homogeneizer (OMNI INTERNATIONAL, Waterbury, CT, USA). Determination of water activity (a_w) was performed using a Decagon CX-1 Water Activity System apparatus (Decagon Devices, Pullman, WA, USA). The titratable acidity was determined following the method described by Zaika, Zell, Smith, Palumbo, and Kissinger (1976) and expressed as a percentage of lactic acid.

Acidity and peroxide values of the fat were determined following the Spanish Official Standards (Presidencia del Gobierno, 1977) after extraction of the fat following the method of Folch, Lees, and Stanley (1957). T.B.A. number was measured according to the method of Tarladgis, Watts, Younathan, and Dugan (1960).

2.3. Statistical methods

Data were subjected to the Student's t test (Steel Torrie, 1960) in order to study significant differences between the different sampling points during the ripening process.

3. Results and discussion

Table 2 summarises the changes in chemical composition during the ripening process of Androlla.

The trend of moisture loss that Androlla undergoes during ripening is similar to other comparable sausages such as 'chorizo' (Barranco Sánchez et al., 1985; Lois, Gutiérrez, Zumalacárregui, & López, 1987) 'salchichón' (Ferrer & Arboix, 1986; Lizaso, Chasco, & Beriain, 1999; Serrano Moreno, 1979) and 'soppressatta molisana'

Table 2
Evolution of the main chemical components during the processing of Androlla (mean of three batches±standard deviation)[a]

	Processing time (days)							
	0	1	3	7	14	21	28	42
Moisture (%)	61.64±0.61a	60.30±1.99ab	57.08±1.84bc	50.71±3.90cd	43.25±5.28d	39.98±8.75de	32.18±5.39e	29.68±9.02e
Protein (N×6.25) (%DM)	33.4±0.06a	32.3±1.25a	35.6±1.79a	33.0±0.54a	35.5±2.90a	34.7±4.54a	32.9±1.34a	35.8±1.97a
Fat (%DM)	40.26±2.64a	41.47±2.76a	43.40±1.83a	42.64±3.94a	41.24±1.51a	42.56±1.45a	43.73±2.49a	41.17±2.11a
Ash (%DM)	7.80±1.08a	7.05±0.50a	7.27±0.61a	7.37±0.16a	6.74±0.75a	7.10±1.32a	7.08±1.20a	7.01±0.56a
NaCl (%DM)	4.23±1.81a	4.17±1.77a	4.48±1.88a	4.14±1.49a	3.63±1.48a	3.62±1.43a	4.12±1.78a	3.64±1.49a
Total carbohydrates (%DM)	0.99±0.42a	0.78±0.38ab	0.70±0.48ab	0.54±0.53ab	0.46±0.37ab	0.37±0.29ab	0.35±0.35ab	0.21±0.19b
Nitrate (ppm)	43.8±17.93a	42.0±18.87a	40.9±18.44a	43.7±25.35a	41.7±29.18a	41.5±30.63a	46.1±27.84a	42.7±24.86a

[a] Means in the same row and parameter group during processing without a common following letter are significantly different ($P<0.05$).

Table 3
Evolution of some parameters of the fat during the processing of Androlla (mean of three batches±standard deviation)[a]

	Processing time (days)							
	0	1	3	7	14	21	28	42
Acidity value (mg KOH / g fat)	1.49±0.29a	1.49±0.28a	1.77±0.23ab	1.86±0.85ab	2.98±0.86abc	3.35±1.51bc	4.50±1.70bc	5.36±1.71c
Peroxide value (meq O_2/ kg fat)	16.09±6.20a	15.42±5.30a	16.78±8.09a	24.84±3.31a	28.28±7.44a	27.00±5.30a	17.55±4.76a	18.94±4.65a
TBA value (mg malonaldehyde/kg)	0.17±0.02a	0.17±0.01a	0.19±0.05a	0.19±0.06a	0.22±0.06ab	0.25±0.06ab	0.29±0.04b	0.40±0.05c

[a] Means in the same row and parameter group during processing without a common following letter are significantly different ($P<0.05$).

(Coppola, Iorizzo, Saotta, Sorrentino, & Grazia, 1997). Barranco Sánchez et al. (1985) reported that the reduction in the moisture contents took place more rapidly than usual and in two other works (Ferrer & Arboix, 1986; Lizaso et al., 1999) moisture contents were higher than in this case during the whole ripening process.

The levels of protein, fat, ash and NaCl observed agree with the results reported elsewhere for this type of product (Lorenzo et al., 2000).

The initial sausage mixture contained 43.8 ppm of nitrates, on average. No significant changes occurred in the quantity of this compound throughout processing which indicates a low nitrate-reducing activity in this sausage during ripening. In 'chorizo', Flores and Alvarruiz (1985) found levels ranging from 567 to 853 ppm. In 'chorizo de Pamplona', Santamaría, Lizárraga, and Astiasarán Bello (1992) found an average concentration of 163 ppm, and in the 'longaniza of Aragón', Marquina, Beltrán, Jaime, Peiró, and Roncalés (1993) showed levels of 120 ppm nitrate. Similar values to ours were described in 'salchichón' (Beriain, Peña, and Bello, 1993; Ferrer & Arboix, 1986) and in 'botillo' (Lorenzo et al., 2000), though low values in 'chorizo of León' have been pointed out (Lois et al., 1987). The low values of nitrate observed by us in Androlla are consistent with the non-addition of nitrate to the mix. The nitrate present may originate from the salt and the spices. Lois et al. (1987) found that paprika and garlic (spices also used in the manufacture of Androlla) show levels of 14 and 286 ppm of nitrate, respectively.

There is little information in the literature about the changes of sugars during the manufacture of ripened sausages (Ferrer & Arboix, 1986; Lizaso et al., 1999). It seems that the degree of degradation is variable, depending on the type of sausage. The data published by Ferrer and Arboix (1986) show that, in the 'salchichón of Vich', the sugars disappear almost completely after two months of ripening, while in 'chorizo of León' (Lois et al., 1987) and in 'salchichón' (Lizaso et al., 1999) considerable levels were detected at its completion. In the case of Androlla, after 24 h, more than 20% of the sugars initially present had disappeared and after 7 days approximately 50%, reaching an average content of 0.21±0.19% of dry matter at the end of ripening. Since the content of added salt in the manufacture of this sausage is low, a rapid development of lactic acid flora, responsible for the degradation of carbohydrates in such a short period would be possible.

The changes in pH and titratable acidity during the ripening of Androlla are shown in Fig. 1. Coinciding with the changes undergone by the total carbohydrates, the pH decreased rapidly in the first seven days from average values of 6.14 to 5.54; this period of time corresponds to the most pronounced development of lactic acid bacteria generally observed in fermented sausages. pH decreased slightly thereafter to average values of 5.47 at day 14 and then increased slightly until the end of ripening, reaching final average value of 5.54. It may be that the pH increase was due to an increase of basic NPN compounds during ripening. The final pH value of

the mature sausages is very variable and, in the bibliography, there are data that fluctuate between 4.42 (Acton & Dick, 1976) and 6.52 (Ferrer & Arboix, 1986). Final pH values shown in Androlla are similar to those observed for other dry-cured sausages (Acton Dick, 1976; Bello, Larralde, & Sáenz de Buruaga, 1974a; Bello, Sáenz de Buruaga, & Larralde, 1974b; Beriain et al., 1993; Casiraghi, Pompei, Dellaglio, Parolari, Virgili, 1996; Domínguez, Ferré, & Zumalacárregui, 1988; Graner, Fonseca, & Basso, 1983; Lee & Styliadis, 1996; León Crespo, Millán, & Serrano Moreno, 1978; Lois et al., 1987; Mateo, Domínguez, Aguirrezábal, & Zumalacárregui, 1996; Santamaría et al., 1992; Serrano Moreno, 1979).

Throughout the manufacture, a gradual increase in lactic acid takes place, more evident in the first 7 days where the initial levels were approximately doubled, reaching an average value of $0.33 \pm 0.12\%$ after 42 days of ripening. Although the minimum values of pH were reached at 14 days of ripening and increased slightly until the end, it is worth pointing out that lactic acid formation continued during the whole process. This lack of correlation between the amount of lactic acid produced and the evolution of pH could be related to the formation of alkaline compounds, as has been suggested by other authors (Cantoni, Bianchi, & Beretta, 1973; Dierick, Vandekerckhove, & Demeyer, 1974). The alkaline compounds produced as a consequence of the protein degradation impede the fall of pH in spite of the lactic acid production. The final lactic acid content was lower than that reported for other sausage varieties (Acton & Dick, 1976; Casiraghi et al., 1996; Domínguez et al., 1988; Santamaría et al., 1992). The non addition of carbohydrates to the mix of Androlla (see Section 2) reduced any significant formation of lactic acid, which would explain the low content of lactic acid found in this work.

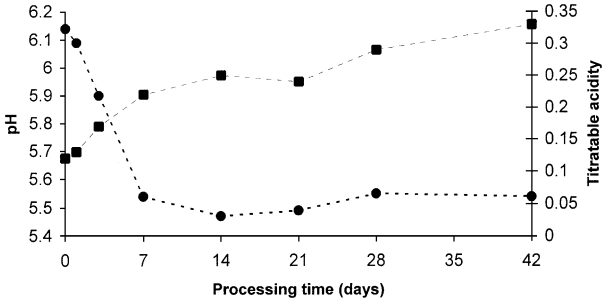

Fig. 1. Changes in pH (●) and titratable acidity (■) (expressed as g of lactic acid/100 g) during the processing of Androlla. Plotted values are the averages of three batches.

The changes in a_w values during the processing are shown in Fig. 2. As expected, the a_w values decreased progressively and constantly throughout the ripening process, from an initial value of 0.977 to 0.883, on average. Values of a_w observed in the final product were almost identical to those reported for 'chorizo of Pamplona' (Santamaría et al., 1992) and 'salchichón' (Beriain et al., 1993; Lizaso et al., 1999), but are substantially higher than those found by other authors for different sausage types (Barranco Sánchez et al., 1985; Chen, Guo, & Liu, 1997; Domínguez et al., 1988; León Crespo et al., 1978; Marquina et al., 1993; Palumbo, Zaika, Kissinger, & Smith, 1976; Serrano Moreno, 1979).

The quantities of NPN, NH_2-N and BVN increased during the ripening as shown in Fig. 3.

NPN increased during the first 28 days and became stable from that time onwards. The increase of this fraction, from 4.52% of total nitrogen to 9.06% (2.13 times), agree with the increase observed by Domínguez and Zumalacárregui (1992–1994) and Lois et al. (1987) in 'chorizo'. However, other authors observed lower increases during ripening of other sausages; Dierick et

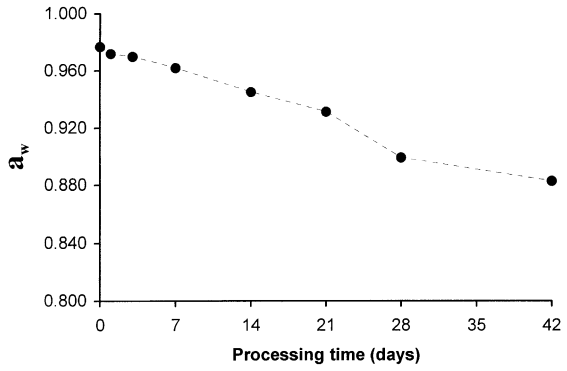

Fig. 2. Changes in a_w during the processing of Androlla. Plotted values are the average of three batches.

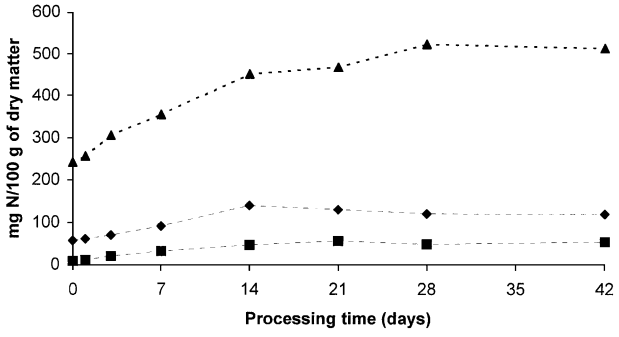

Fig. 3. Changes in non-protein nitrogen (NPN) (▲), α-aminoacidic nitrogen (NH_2-N) (◆) and total basic volatile nitrogen (BVN) (■) contents during the processing of Androlla. Plotted values are the average of three batches.

al. (1974), León Crespo et al. (1985), Ferrer and Arboix (1986), DeMasi, Wardlaw, Dick, and Acton (1990) and Lizaso et al. (1999) reported an increase of nearly 50% in different dry-cured sausages and Lois et al. (1987) reported increases of 10% for 'chorizo' manufactured with the addition of sugars to the mix.

The average final content of NPN (514 mg/100 g of dry matter) is into the range of values observed for other sausage varieties (Acton & Dick, 1976; DeMasi et al., 1990; Dierick et al., 1974; Domínguez et al., 1988; Ferrer & Arboix, 1986; León Crespo et al., 1985; Lois et al., 1987; Marquina et al., 1993; Santamaría et al., 1992) and indicates that the degree of proteolysis undergone by Androlla during ripening is of the same order as in these sausages.

The amount of NH_2-N increased during the first 14 days of ripening, then stabilized and showed a tendency to decrease at the end of ripening. A substantial initial increase in NH_2-N content was also reported by Dierick et al. (1974), Ferrer and Arboix (1986) and Mateo et al. (1996) during the ripening of different sausage varieties. The level of α-aminoacidic nitrogen found in the mix of Androlla (0 days of ripening; 57 mg/100 g of dry matter on average) is low when compared with values in the literature (Bello, Saenz de Buruaga, et al., 1974b; Domínguez & Zumalacárregui, 1992–1994; Ferrer & Arboix, 1986; Mateo et al., 1996). In the same way, the levels shown at the end of the ripening (119 mg/100 g of dry matter on average) are very much below the values observed in these studies in other ripened sausages.

Likewise, the ripening process caused an increase in the total basic volatile nitrogen (Fig. 3). The average initial content of BVN (9.05 mg/100 g of dry matter) is rather lower than that observed in 'chorizo of León' (19.1–37.7 mg/100 g of dry matter; Domínguez Zumalacárregui, 1992–1994) and in 'chorizo de cebolla' from Galicia (35 mg/100 g of dry matter; Salgado, Rodríguez, López, & Carballo, 1999). In this study final values (53.9 mg/100 g of dry matter) are within the range of values found in 'Botillo' by Lorenzo et al. (2000), but are lower than those observed by Domínguez and Zumalacárregui (1992–1994) and by Salgado et al. (1999) in other sausage types. However, the increase we observed for BVN during the ripening (about 6 times) was higher than that reported by the earlier mentioned authors, which indicates an intense degrading activity of the amino acids during the ripening of Androlla.

The lipolytic and oxidative changes are shown in Table 3. The free fatty acid content increased 3.6-fold with time from 1.49 mg of KOH/g of fat in the mix to 5.36 mg of KOH/g of fat after 42 days of ripening, an increase which is similar to that quoted by Domínguez (1988) in artisanal 'chorizo'. However, other authors have found higher increases. León Crespo et al. (1985) and Domínguez (1988) have described increases of 6 to 7 fold in 'chorizo', while Salgado et al. (1999) and Ferrer and Arboix (1986) have obtained increases of 10- to 11-fold in 'chorizo de cebolla' and in 'salchichón of Vich', respectively.

Our initial values of acidity of fat are in the range of those observed for other dry-fermented sausages (Domínguez et al., 1988; Ferrer & Arboix, 1986; León Crespo et al., 1985; Nagy, Mihályi, & Incze, 1989; Roncalés, Aguilera, Beltrán, Jaime, & Peiró, 1991). The final values observed after 42 days of ripening are also within the broad range of values found in the literature.

The intensity of lipolysis is different in the distinct dry cured sausages and Androlla undergoes a moderate lipolysis throughout ripening.

The peroxide value (initial values of 16.1 ± 6.20 meq of 0_2/kg of fat) underwent a rapid increase from the third day of the manufacture process, reaching values of 28.3 ± 7.44 meq after 14 days, remaining constant until the 21st day, and decreasing during the final phase of ripening, reaching levels close to the initial ones. During ripening of some sausages, PV increases (Ferrer & Arboix, 1986; Salgado et al., 1999), in others it remains constant (Salgado et al., 1999) or decreases (Nagy et al., 1989). The initial values found in Androlla (16.1 ± 6.20 meq) were higher than the initial figures found by other authors in other dry-fermented sausages (Ferrer & Arboix, 1986; Nagy et al., 1989), which shows that autooxidation reactions had already started before stuffing. The final PV values found in this study (18.9 ± 4.65 meq of 0_2/kg of fat) are higher than those usually found in the literature (Ferrer & Arboix, 1986; Nagy et al., 1989; Santamaría et al., 1992) though similar to those described by Beriain et al. (1993) in 'salchichón' and by Rodríguez, Salgado, López, and Carballo (1999) in 'chorizo de cebolla', 'chorizo rosario' and 'botillo'. The high peroxide values in Androlla may be due to the fact that autooxidation had already begin in the mix. Furthermore, the low content of nitrate found in this sausage and its non degradation throughout ripening could be of influence since, as is well known, the nitrites are powerful antioxidants (Cross & Ziegler, 1965; Watts, 1954). Finally, the presence of bones inside the sausage, and the fact that the processes of mixing and stuffing are not carried out under vacuum conditions, are decisive factors for the presence of oxygen in high concentrations inside the sausage, which favours the development of autooxidation reactions.

The T.B.A. value increased gradually during processing. The values that Androlla shows for this parameter, both in the initial phase and in the final product, are lower than those found in the literature for other dry-fermented sausage varieties (Domínguez, 1988; Nagy et al., 1989; Rodríguez et al., 1999; Salgado et al., 1999; Santamaría et al., 1992). However the observed increase during ripening (2.35-fold) is higher than that found by other authors (Domínguez, 1988; Nagy et al., 1989).

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