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Relationship between biochemical and sensory quality characteristics of different commercial brands of salchichon

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Abstract

The nitrogen fractions (myofibrillar, sarcoplasmic, denatured and non-protein), free amino acid composition, parameters related to fat (iodine index, carbonyl compounds, peroxide value and free fatty acids composition), pH, water activity (a_w), moisture and sensory quality characteristics were measured in three commercial brands of salchichon (A, B, C) manufactured in three different industries in order to study the relationship between their biochemical characteristics and their organoleptic quality.

The different characteristics and composition of the lipid, nitrogen fractions and free amino acid composition in the three commercial brands led to differences in the organoleptic quality, specially in the texture. The best texture scores were related to a higher insolubilisation of the myofibrillar fraction and a higher water content. The panellists did not notice differences in the flavour among the sausage brands probably due to the addition of black pepper and different spices in the initial mix.

Keywords: Salchichon; Biochemical characteristics; Sensory properties

1. Introduction

The production of cured dry sausages is of great importance to the meat industry of Mediterranean countries and Germany. In Spain, one-fifth of the total meat products manufactured are cured dry sausages (Fernandez et al., 1995). Salchichon is a typical Spanish dry fermented sausage. Its formulation is characterised by the presence of different proportions of black pepper.

The production of salchichon implies, in a general way, three well-defined phases: mixing of ingredients, fermentation and drying. Physical, microbiological and biochemical changes (Fernandez et al., 1995), involving tissue enzymes as well as microbial enzymes, take place in sausages during fermentation and drying. These changes are influenced by the raw material characteristics (Bacus, 1984) and the process conditions (Beriain, Peña & Bello, 1993) and they will be reflected in the organoleptic properties of the final product. Thus, proteolysis and protein insolubility will influence the flavour and texture of the final product (Whiting, 1988). The release of free amino acids are highly correlated with flavour development (Mc Lain, 1968; Córdoba et al., 1994) and they have been reported as precursors of sour, sweet, and bitter taste (Kato, Rhue & Nishimura, 1989). Other types of changes that can occur in meat products are lipolytic and oxidative changes, that release free fatty acids and carbonyl compounds, and they also have a direct influence on the development of flavour and aroma (Demeyer, Hooze & Mesdom, 1974; Shamberger, Shamberger, & Willios, 1977; León-Crespo & Millán, 1977; León-Crespo et al., 1985).

The determination of parameters such as moisture, fat content, sugars, protein and salt allow the characterisation and classification of meat products into commercial categories in the Spanish Regulations. However, these specifications are not enough to describe the dry cured products in relation to their organoleptic characteristics. So, it is important to study how the differences in the composition of the lipid and nitrogen fractions content of sausages can influence on their organoleptic characteristics and how they are detected by consumers.

This work covers the study of the lipid, nitrogen fractions and free amino acids content of three commercial brands of salchichon and their relation to the organoleptic characteristics (flavour and texture) evaluated by a laboratory panel.

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2. Materials and methods

2.1. Materials

Three commercial brands of salchichon (A, B and C) from three different sausage companies were selected.

The ingredients used to produce the salchichon brands are shown in Table 1. The process made in the three products was the following: frozen pieces of pork and beef were mixed with salts and spices in a cutter. Frozen pork fat was added after being minced in a 3 mm diameter mincer. The mixture was vacuum minced and stored at 4°C for 20 h. It was then stuffed in a 60 mm diameter sausage cellulose casing, fermented for 3–5 days (25°C, 90% RH) and dried for 4 weeks (15–18°C, 75–80% RH). Sausages were analysed at the final product (4th week of drying). The analysis of four sausages from each brand (A, B, C) was carried out in duplicate.

2.2. Methods

2.2.1. General parameters

Water activity (a_w) using an Aqualab CX2 instrument. pH (ISO R 2917-1974) with an Orion Research potentiometer for solid samples. Moisture (ISO 1442-1973a). Chloride content (AOAC, 1984).

2.2.2. Parameters related to proteins

The different nitrogen fractions (sarcoplasmic, myofibrillar, denatured and non-protein) were separated according to Helander's method (1957), modified by Bello, Larralde and Saenz de Buruaga (1974). The nitrogen concentration of each fraction and the total nitrogen in the samples were then determined using a Kjeldahl method (ISO R 937-1981). Total protein was calculated as the total nitrogen × 6.25. The sarcoplasmic and myofibrillar fraction were expressed as the percentage of the total nitrogen, denatured and non-protein were expressed as mg/g of nitrogen in each fraction.

Table 1
Composition of different commercial brands of salchichon (A, B, C)

	A	B	C
Lean pork (%)	55	50	40
Beef (%)	25	30	20
Pork fat (%)	20	25	30
Common salt (g/kg)	20	20	20
Sugars (g/kg)	15	15	15
Black pepper (g/kg)	2	2	2
Plyphosphate (g/kg)[a]	2	2	2
Sodium ascorbate (g/kg)	0.3	0.3	0.3
Nitrites (g/kg)	0.2	0.2	0.2
Nitrates (g/kg)	0.1	0.1	0.1

[a] Sodium pyrophosphate and potassium metaphosphate.

2.2.3. Free amino acids analysis

The preparation of amino acid extracts was made according to the method described by Aristoy and Toldrá (1991). 4 g of salchichon was diluted (1:5)(w/v) with 0.1 N HCl, and homogenised in a stomacher (LAB-BLENDER, 400). 20 ml of Methionine sulphone was added to the mix. Supernatant was filtered and deproteinised by adding 2.5 volumes of acetonitrile. Deproteinised samples were centrifuged at 15,000 rpm for 5 min and freeze-dried overnight. Free amino acids were derivatised with phenylisothiocyanate using the Waters PICO-TAG method: Lyophilised samples received 20 μl of amino acid elution solution (6:2:2 ratio of H_2O: triethylamine: methanol) and were freeze-dried again. Dried samples then received 20 μl of derivatisation solution (7:1:1:1 ratio of methanol : triethylamine : phenylisothiocyanate : H_2O) and were incubated for 30 min at room temperature before freeze-drying. Samples were resuspended in a phosphate buffer containing 5% acetonitrile and were filtered through a 0.45-μm nylon filter (Millipore). Samples were analysed on a Waters HPLC system (Millipore/Waters, Mississauga, ON) consisting of two 6000A pumps, a 440 Fixed-Wavelength Absorbancy Detector set at 254 nm, a Temperature control module, a 710B WISP™ Sample Processor and a 840 Data and Chromatographic Control Station. The column used was a Waters Pico-tag™ C_{18} reverse phase column maintained at 46°C. A gradient with two solvents was used: (a) 70 mM sodium acetate adjusted to pH 6.55 with acetic acid and added with 5% acetonitrile, and (b) 45% acetonitrile 40% water and 15% methanol. Peak identification and quantification were accomplished by determining retention times and recoveries of free amino acids standards (Sigma Chemical Company, St Louis, MO).

2.2.4. Parameters related to lipids

Total fat content were determined using the Soxhlet method (ISO 1443-1973b). The lipid fraction was extracted from the product according to the method of Bligh and Dyer (1959). Iodine value (ISO, 1979), acidity value (ISO, 1980), carbonyl compounds (Henick, Benca & Mitchell, 1954) and peroxide index (ISO, 1977) were determined. Free fatty acids in the lipid fraction were separated by thin layer chromatography, dissolved in *n*-hexane, filtered and methylated (Eichhorn, Bailey & Blomquist, 1985). Methyl esters were analysed on a Hewlett-Packard gas chromatograph (HP-5890) equipped with a hydrogen flame ionisation detector. Separations were effected with an HP-FFAP capillary column (25 m × 0.2 mm i.d. × 0.3 μm) under the following conditions: (a) carrier gas: helium at 1 ml/min, (b) oven temperature: 180–210°C at 3°C/min, 210°C for 5 min, 210–225°C at 5°C/min, 9 min at 225°C, (c) injector temperature: 230°C, (d) detector temperature: 240°C. On injection, the stream of helium and the injected

volume of the sample were split (1:24). Methyl ester standards of fatty acids (Matreya Inc.) were used for the peaks' identification. Results were expressed as percentage.

2.2.5. Sensory evaluation

The sensory analysis of the samples was carried out by a preference ranking test. The laboratory panel was composed by fifteen people consisting of faculty and staff of Public University of Navarra, and ranged in age from 20 to 35. Each panellist was presented two slices of each brand about 2 mm in thickness, obtained with a slicing machine and inmediately served at room temperature. Testing took place in a sensory panel room equipped with fluorescent lights. Apple slices were allowed between samples. Panellists ranked the sausage brands in preference order as far as the tested parameters were concerned. The parameters evaluated were related both to the technological process (presence of crust and mincing diameter) and to the sensory quality (colour, flavour, aroma, texture and overall acceptability).

Four sausages of each brand were used and the parameters of each sample were determined in three replicates.

2.2.6. Statistical analysis

One way analysis of variance and multiple comparison test of Tuckey were conducted to test significance among commercial brands. A Friedman test was applied to the sensorial data (Friedman, 1937). A factorial analysis was used to study the relationship between the sensorial and the instrumental data. The SPSS statistic program was used (SPSS, 1.2, 1998).

3. Results and discussion

Table 2 shows the average values of the general parameters which characterise A, B and C sausages: a_w of about 0.9, pH between 4.87 and 4.62 and 38% approximate moisture. These results comply with current requirements under Spanish Food Legislation and agree with those from other authors (Melgar, Sanchez Monge & Bello, 1990; Santamaría, Lizarraga, Astiasarán & Bello, 1992; Beriain et al., 1993; Chasco, Beriain & Bello, 1993). However, significant differences ($p < 0.001$) were observed among them, that is, sausage A presented the higher pH and lower a_w values and C brand showed the lowest moisture and chloride content.

The solubilised protein fraction, influenced by the insolubility and proteolysis phenomena that take place during the manufacturing process, has a great importance in the texture of cured dry sausages (Deketelaere, Demeyer Vadereremhoue & Vervaere, 1974). The sarcoplasmic solubility (Table 3) was slightly lower in B brand sausages (22%). The myofibrillar solubility percentage showed higher values in C sausages than in A and B brands, that did not present significant differences between them. The myofibrillar solubility was lower than the sarcoplasmic nitrogen solubility in all of the studied samples.

The major proteolytic activity, evidenced by a greater non-protein nitrogen content (Table 3) and total free amino acids concentration (Table 4), was observed in sausage A. The results found in B and C brands were similar to those reported by other authors for low pH sausages (Lois, Gutiérrez, Zumalacarrégui & López, 1987; Johansson, Berdagué, Larsson, Tran & Borch, 1994). Differences found between A and B–C brands in protein solubility and non-protein nitrogen could be due to the higher pH showed by A brand (Table 2). Verplaetse, De Bosschere and Demeyer (1989) pointed out that the pH values of 4.5–5.0 and the temperature of ripening of 15–20°C were optimal for the activity of proteolytic enzymes. In Table 4, it can be observed that about four and seven amino acids accounted for 57–58% of the total concentration of free amino acids in each group of sausages. The concentrations of most of the amino acids shown in Table 3 were higher in brand A than the other brands, B and C, and than the concentrations reported by Langner (1969), Dierick, Vandekerckhove and Demeyer (1974) and De Masi (1990). Muscle aminopeptidases except leucyl aminopeptidase may contribute to the generation of free amino acids specially during the fermentation stage where pH is not so low yet and temperature is around 25°C (Flores, Sanz & Toldrá, 1998). These conditions of pH value, could be

Table 2
General parameters of three commercial brands of salchichón (A, B, C)[A]

	A	B	C	
Water activity	0.894 ± 0.001^a	0.900 ± 0.001^b	0.904 ± 0.002^b	***
pH	4.87 ± 0.11^a	4.62 ± 0.01^b	4.66 ± 0.01^b	***
Moisture (%)	39.49 ± 0.34^a	38.52 ± 0.14^b	37.29 ± 0.45^b	***
NaCl (%)	7.36 ± 0.05^a	6.65 ± 0.12^b	5.78 ± 0.09^c	***

[A] The percentage of NaCl and total and soluble sugar are refer to dry sample. Any two means followed by the same superscript are not significant different ($p > 0.05$); (***$p < 0.001$).

Table 3
Parameters related to proteins in three commercial brands of salchichón (A, B, C)[A]

	A	B	C	
Total protein (%)[B]	33.59 ± 0.37^{a}	33.64 ± 0.67^{a}	28.92 ± 0.55^{b}	***
Myofibrillar solubility (%)[C]	11.63 ± 1.00^{a}	11.43 ± 0.67^{a}	20.75 ± 1.28^{b}	***
Sarcoplasmic solubility (%)[C]	25.82 ± 0.48^{a}	21.96 ± 1.33^{b}	24.70 ± 0.84^{ab}	*
Nonprotein nitrogen (mg/g)	8.76 ± 0.18^{a}	5.32 ± 0.67^{b}	6.67 ± 0.12^{c}	***
Denatured nitrogen (mg/g)	19.28 ± 0.97^{a}	19.41 ± 1.20^{a}	14.69 ± 1.00^{b}	***

[A] All the values are referred to dry sample. Any two means followed by the same superscript are not significantly different ($p > 0.05$) ($*p < 0.05$; $***p < 0.001$).
[B] Total protein is obtained as total nitrogen × 6.25.
[C] Myofibrillar solubility is obtained as myofibrillar nitrogen/total nitrogen × 100; Sarcoplasmic solubility is obtained as sarcoplasmic nitrogen/total nitrogen × 100.

Table 4
Free amino acids concentration (mg/100 g dry matter) found in different commercial brands of salchichon (A, B, C)[A]

	A	B	C	
Aspartic acid	22.97	28.02	18.98	ns
Glutamic acid	886.85^{a}	267.64^{b}	249.78^{b}	***
Serine	45.41^{a}	27.23^{b}	21.55^{b}	*
Asn	66.80^{a}	28.12^{b}	22.18^{b}	**
Glycine	69.59^{a}	25.69^{b}	23.61^{b}	***
Taurine	81.92^{a}	26.71^{b}	29.20^{b}	**
Threonine	34.11	27.77	24.39	ns
Alanine	216.36^{a}	82.11^{b}	73.93^{b}	***
Carnosine	206.26	197.03	58.67	ns
Proline	53.50	51.31	44.41	ns
3Metil-Hystidine	23.60^{a}	33.27^{a}	7.79^{b}	*
α-amino butiric	16.26^{a}	29.76^{a}	4.08^{b}	***
Tyrosine	38.14^{a}	13.52^{b}	7.62^{b}	***
Valine	158.46^{ab}	182.08^{b}	132.14^{a}	*
Metionine	60.12^{ab}	71.84^{b}	48.96^{a}	*
Cysteine	45.54^{a}	212.47^{b}	40.36^{a}	**
Isoleucine	132.66^{a}	180.51^{b}	105.10^{a}	**
Leucine	305.41	363.12	283.27	ns
Phenylalanine	45.49^{a}	190.45^{b}	147.05^{b}	***
Triptophan	43.59	7.20	19.08	ns
Hydroxilysine	21.77	41.02	30.29	ns
Ornitine	11.99^{a}	6.34^{b}	7.61^{b}	*
Lysine	34.04	37.42	15.23	ns
Total free amino acids (mg/g dry matter)	24.43^{d}	19.04^{c}	15.20^{b}	***

[A] $*p < 0.05$; $***p < 0.001$, ns non-significative. The same superscript means no significant differences ($p > 0.05$).

the origen of the high concentration of free amino acids in brand A determining the best overall acceptability by the panel. Glutamic acid, alanine, carnosine, valine, isoleucine and leucine were the predominant amino acids in brand A; glutamic acid, carnosine, valine, cisteine, isoleucine, leucine, and phenylalanine, in B brand; and glutamic acid, valine, isoleucine, leucine and phenylalanine in C brand. The more abundant free amino acids detected in fermented sausages and presented in the three brands were glutamic acid, leucine, valine and isoleucine which showed values higher than 1 mg/g of dry matter. Thus these amino acids could be responsible for salchichon characteristic flavour. The differences among the three studied salchichon brands could be attributed to alanine, carnosine, cisteine and phenylalanine because they are majority amino acids and they are presented in different concentrations in the three brands. The majority of the above-stated amino acids were recorded as having "bitter" flavour characteristics (Kato et al., 1990). Arginine was not detected in any of the bands, possibly due to the non-addition of garlic to salchichon, which is an important source of taste compounds, specially of Arginine (Mateo, Dominguez, Aguirrezábal & Zumalacárregui, 1996).

The lipolytic phenomena that took place during curing of sausages were related to the acidity value (Table 5), being brand C the one that presents the less value, while A and B sausages showed similar values

between them. These modifications of the lipid fraction, very important in the development of the aroma (Wurziger & Ristow, 1966; Cantoni, Molnar, Renon & Giolitty, 1967), involve an increase on free fatty acids due to the microbial activity, specially Micrococci (Smith & Alford, 1969).

The unsaturation of the lipid fraction, reflected by the iodine value, was different in all of the three sausage brands studied. This fact is also reflected in the fatty acid composition (Table 6). Sausages of brand B presented a higher content on saturated fatty acids (myristic ($C_{14:0}$), palmitic ($C_{16:0}$) and stearic ($C_{18:0}$)) and lower percentages of unsaturated fatty acids (oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$)). Besides, the lower susceptibility to oxidation of the saturated portion of lipids is also made evident by a lower carbonyl compounds content, that confer these products their characteristic flavour (Anderson, 1980; Mottram & Edwards, 1983; Melgar et al., 1990).

The differences in the lipolytic and proteolytic phenomena found among commercial products might be due to the different technology applied by each industry and to the influence of the raw materials and, thus, to the feeding of the animal source (Chasco et al., 1993). Besides, the studied brands contained different proportions of some spices added to the mix (black pepper), various compounds with prooxidant power such as salt, haeme and non-haeme iron, peroxides produced by micro-organisms (*Lactobacillus* spp.) and different proportions of the oxygen that remains in the core of the sausage (Domínguez & Zumalacárregui, 1991).

The sensory evaluation of the three commercial brands studied pointed out significant differences ($p < 0.05$) in the following parameters: mincing diameter, presence of crust, colour and texture. These results could be explained by the different amounts of fat in the three products. The C brand had a greater amount of fat determining the lowest scores in the characteristics related to texture. The flavour and the overall acceptability had similar scores in the three commercial brands of sausage. These results suggested that the use of different technologies and different raw materials proportions had an influence mainly on aspects related to the mincing type and diameter, texture and consistence, but it had no specific effect on flavour as it has been shown in the present study in sausages with similar time of curing. Thus, differences in the oxidative and proteolytic phenomena of the three brands did not produces flavour differences noticed by the panellists. Nevertheless, the variations in the protein fraction did have an influence on the final texture of the product.

A factorial analysis was applied to the texture sensory data and the overall acceptability, the nitrogen fractions (myofibrillar, sarcoplasmic, denatured, non-protein nitrogen) and the pH and moisture. The first two principal factors explained the 75% of the total variance. As it is shown in Fig. 1, overall acceptability was related to texture, which implies that it was scored by the panellists as being one important factor of sausage quality. The best texture scores were related to a higher insolubilisation of the myofibrillar fraction and were favoured by a higher pH and water content. These characteristics

Table 5
Parameters related to fat characteristics and stability in three commercial brands of salchichón (A, B, C)[A]

	A	B	C	
Total fat (%)	51.11 ± 0.23^{a}	54.70 ± 0.30^{b}	58.66 ± 0.49^{c}	***
Iodine value (%I_2)	71.49 ± 0.61^{a}	69.14 ± 0.49^{b}	64.76 ± 0.40^{c}	***
Acidity value (mg KOH/g fat)	17.00 ± 0.59^{a}	17.09 ± 0.28^{a}	14.37 ± 0.23^{b}	***
Peroxide value (meq O_2/kg fat)	56.84 ± 1.64	54.88 ± 0.39	54.21 ± 1.89	ns
Carbonyl compounds (μmol CO/g fat)	17.09 ± 0.24^{a}	15.07 ± 0.90^{a}	28.54 ± 4.23^{b}	**

[A] Any two means followed by the same superscript are not significant different ($p > 0.05$); (ns = no significance; $**p < 0.01$; $***p < 0.001$).

Table 6
Principal free fatty acids composition (% relative) of three commercial brands of salchichón (A, B, C)[A]

	A	B	C	
Myristic ($C_{14:0}$)	1.15 ± 0.02^{a}	1.79 ± 0.09^{b}	1.29 ± 0.14^{a}	***
Palmitic ($C_{16:0}$)	20.67 ± 0.14^{a}	23.68 ± 0.25^{b}	21.24 ± 0.67^{a}	***
Stearic ($C_{18:0}$)	12.68 ± 0.33^{ab}	13.57 ± 0.43^{b}	12.07 ± 0.15^{a}	*
Oleic ($C_{18:1}$)	40.71 ± 0.11^{a}	38.95 ± 0.26^{b}	40.43 ± 0.50^{a}	***
Linoleic ($C_{18:2}$)	14.07 ± 0.13^{a}	11.72 ± 0.17^{b}	13.41 ± 0.59^{a}	**
Linolenic($C_{18:3}$)	0.99 ± 0.03^{a}	*traces*	1.23 ± 0.07^{b}	**
$C_{16:0}/C_{16:1}$	9.42 ± 0.07^{a}	10.56 ± 0.16^{b}	9.91 ± 0.06^{c}	***
$C_{18:0}/C_{18:1\pm}C_{18:2\pm}C_{18:3}$	0.23 ± 0.01^{a}	0.27 ± 0.01^{b}	0.22 ± 0.01^{a}	***
Saturated/insaturated	0.58 ± 0.01^{a}	0.70 ± 0.01^{b}	0.59 ± 0.02^{a}	***

[A] Any two means followed by the same superscript are not significant different ($p > 0.05$); ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$).

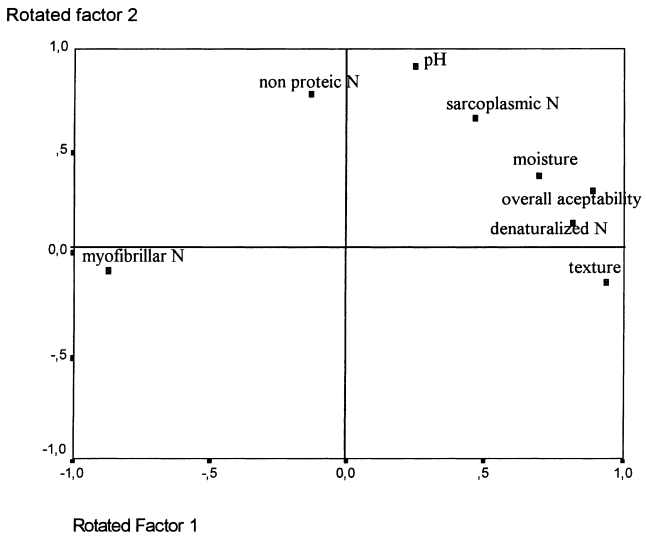

Fig. 1. Factorial analysis of some parameters related to proteins and sensorial scores (two first principal rotated factors).

were shown by the A brand determining the best sensory quality.

In conclusion, the control of proteolysis phenomena is very important to guarantee the homogenity of the organoleptic characteristic of texture in cured products for them to be the best scored by consumers.

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