



Preliminary Assessment of Microbiological and Physicochemical Properties of Artisanal “Chouriço de Carne” Fermented Sausages, Manufactured in Northern Portugal [†]

Ana Sofia Faria ^{1,2} , Gisela Rodrigues ^{1,2}, Rebeca Becker Miranda ^{1,2}, Olga María Bonilla-Luque ³, Laís Carvalho ^{1,2}, Nathália Fernandes ^{1,2} , Miguel Angel Prieto ^{4,*} , Vasco Cadavez ^{1,2} and Ursula Gonzales-Barron ^{1,2,*}

¹ Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; anafaria@ipb.pt (A.S.F.); giselar0608@hotmail.com (G.R.); rebecabeckermiranda@hotmail.com (R.B.M.); laismagalhaescarvalho@hotmail.com (L.C.); nathaliaraquelx@gmail.com (N.F.); vcadavez@ipb.pt (V.C.)

² Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

³ Department of Food Science and Technology, UIC Zoonosis y Enfermedades Emergentes (ENZOEM), CeIA3, Universidad de Córdoba, Campus Rabanales, 14014 Córdoba, Spain; v32boluo@uco.es

⁴ Nutrition and Bromatology Group, Faculty of Food Science and Technology, University of Vigo, Ourense Campus, 32004 Ourense, Spain

* Correspondence: mprieto@uvigo.es (M.A.P.); ubarron@ipb.pt (U.G.-B.); Tel.: +34-654694616 (M.A.P.); +351-273-303-325 (U.G.-B.)

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Abstract: “Chouriço de carne” is a Portuguese ready-to-eat dry fermented sausage which relies on effective spontaneous fermentation to ensure its microbial safety and desired organoleptic properties. This study aimed to assess selected microbiological and physicochemical characteristics of artisanal chouriço produced by 14 different regional producers. Aerobic mesophilic bacteria, lactic acid bacteria, *Staphylococcus aureus*, *Clostridium* spp., *Listeria* spp. and *Salmonella* spp., in addition to pH, water activity and moisture, were evaluated for each of 70 samples. Principal Component Analysis of all these attributes was performed to build quality maps of the analyzed lots. The results showed great variability between sausages of different producers within the same geographic region, and *S. aureus*, *Clostridium* spp., *Salmonella* spp. and *Listeria* spp. were identified in sausages of several producers, highlighting the need for stricter microbiological control and standardization of production processes among artisanal producers of chouriço.

Keywords: *Clostridium*; *Staphylococcus aureus*; *Salmonella*; *Listeria*; pathogens; lactic acid bacteria; pH; moisture; principal component analysis; fermented sausage

1. Introduction

“Chouriço de carne” is a Portuguese ready-to-eat dry fermented sausage, manufactured predominantly in the Trás-os-Montes and Alentejo regions, where it is a staple food in the daily diet of rural populations. Generally, this type of sausage is produced with roughly chopped pork meat and fat, seasoned/marinated with salt, wine, water, garlic, sweet and/or spicy paprika and bay leaf. This mixture is left to rest at low temperatures for up to 4 days; it is then filled in dry natural casings (pre-rehydrated) and hung to naturally ferment (without the aid of starter cultures) and dry by means of an intermittent smoking process for several weeks. “Chouriço de carne” is still produced in an artisanal manner in certain regions of the interior of Northern Portugal and can present extensive nutritional,

organoleptic, physicochemical and microbiological diversity, depending on the raw materials and the production processes of each producer, as well their standardization and hygiene practices [1–3].

This study aimed (i) to assess the variability of selected microbiological and physicochemical attributes of chouriço manufactured by different artisanal producers representative of the northeastern Portugal region and (ii) to comprehend the associations between the studied properties through quality maps generated by principal component analysis.

2. Materials and Methods

Five samples of finished “chouriço de carne” from the same lot of production were purchased from 14 artisanal producers situated in the counties of Bragança, Carraceda de Ansiães, Mirandela, Mogadouro, Miranda do Douro, Vila Flor, Vimioso and Vinhais, located in the Bragança district, northeastern Portugal. Samples were transported to and stored in refrigeration at the laboratory until analysis. Under aseptic conditions, the surface of the sausage was cleaned, the casing removed and its contents portioned for physicochemical and microbiological analysis.

2.1. Physicochemical Analysis

The physicochemical properties measured were pH, water activity (a_w) and moisture. For pH measurement, 10 g of sample was homogenized (Interscience Bag Mixer 400, France) in 90 mL of Buffered Peptone Water (611014 Liofilchem, Italy) and the homogenate's pH was measured in triplicate using a pH meter (FiveGo F2, Mettler-Toledo, Switzerland) equipped with an LE438-IP67 electrode. To measure a_w , a portion of the ground sample was pressed to fit into a disposable cuvette into an Aqualab 4TE water activity meter (4TE Decagon, Pullman, WA, USA, sourced in Portugal) and the values recorded in duplicate. Moisture was determined according to ISO standards [4].

2.2. Microbiological Analysis

Microbiological analyses included the enumeration of mesophilic bacteria, lactic acid bacteria (LAB), presumptive *Clostridium perfringens*, *S. aureus* and *Listeria* spp., and the detection of *Salmonella* spp. and *Listeria* spp. as well. For microbiological analyses, 25 g of chouriço was homogenized in 225 mL of Buffered Peptone Water (611014 Liofilchem, Roseto, Italy) for 90 s (Interscience Bag Mixer 400, Le Bourg, France). For enumeration (i) of mesophiles, 1 mL aliquots from decimal dilutions were incorporated in Plate Count Agar (610040, Liofilchem, Roseto, Italy) [5]; (ii) for lactic acid bacteria, 1 mL aliquots were plated by incorporation in MRS and M17 Agar (610024 and 610192, Liofilchem, Roseto, Italy) and cultured in anaerobiosis [6]; (iii) for presumptive *Clostridium perfringens*, 1 mL aliquots were incorporated into TSC Agar (DSHB3042, Alliance Bio Expertise, Bruz, France) supplemented with Egg Yolk Emulsion (80219, Liofilchem, Roseto, Italy) and D-Cycloserine (DSHB3021, Alliance Bio Expertise, Bruz, France) and cultured in anaerobiosis [7]; (iv) for *S. aureus*, 0.1 mL aliquots were spread-plated on Egg Yolk Tellurite Emulsion supplemented (80122, Liofilchem, Roseto, Italy) Baird-Parker Agar (610004, Liofilchem, Roseto, Italy) [8]; (v) for *Listeria* spp. enumeration, 0.1 mL volume was spread onto *Listeria* Oxford Agar (610167, Liofilchem, Roseto, Italy) enriched with *Listeria* Oxford Supplement (81027, Liofilchem, Roseto, Italy) [9]. For the detection of *Listeria* spp. and *Salmonella* spp. in 25 g of sample, the respective ISO standards were applied [10,11]. Presumptive *Listeria* spp. colonies were confirmed using the *Listeria* System 18R kit (71640, Liofilchem, Roseto, Italy) and suspect *Salmonella* spp. colonies were confirmed by *Salmonella* Latex Kit (96151, Liofilchem, Roseto, Italy). All plating was performed in duplicate (*Listeria* spp. counts in triplicate), and colony-forming units were converted to log CFU/g.

2.3. Statistical Analysis

To synthesize the information contained in the 10 physicochemical and microbiological parameters analyzed, and to understand their interrelationships, the data were submitted

to Principal Component Analysis (PCA) using the R software (version 4.3.1, R Foundation for Statistical Computing, Austria). The analysis was conducted using the function *prcomp()* and the library *factoextra*, resulting in a varimax-rotated solution for three factors. Using the three-dimensional PCA, quality maps of the microbiological and physicochemical attributes of chouriço were generated by projecting the scores of the samples onto the length of the principal components, and the scores were grouped by producer (i.e., county origin).

3. Results and Discussion

The microbiological and physicochemical quality of “chouriço de carne” sausages produced in an artisanal manner in northeastern Portugal exhibited substantial variability, as observed from the producer-specific mean values for counts of total mesophiles (5.301–9.013 log CFU/g), LAB counts (7.151–11.405 log CFU/g), presumptive *C. perfringens* (<0.699–2.504 log CFU/g), *S. aureus* counts (<1.699–4.000 log CFU/g), pH (4.707–6.230), a_w (0.7717–0.972) and moisture (11.86–50.23%). While *Listeria* spp. was never enumerated, it was detected from one of the fourteen sampled producers at an incidence of 1 (five positive samples out of five tested from producer “MirandaDoDouro”) and *Salmonella* spp. was detected in one producer at an incidence of 0.40 (two positive samples out of five tested samples from producer “VilaFlor”).

The contribution of the sausages’ quality variables to the principal components can be assessed through their correlations to each of the extracted components (Table 1). Three components were extracted, which account for 63% of the variability observed in the 10 quality attributes studied.

Table 1. Correlation coefficients of the tested physicochemical and microbiological attributes of “Chouriço de carne” sausage, with the three Varimax-rotated factors (PC1, PC2 and PC3), alongside communalities and their explained variances.

Variable	PC1	PC2	PC3	Communalities
pH	−0.85	−0.14	0.14	1.1
a_w	0.87	0.08	0.24	1.2
Moisture	0.84	0.10	−0.10	1.1
<i>Listeria</i> spp.	−0.51	0.33	0.05	1.7
<i>Salmonella</i> spp.	0.19	0.36	0.06	1.6
Mesophiles	0.25	0.79	0.24	1.4
<i>Staphylococcus aureus</i>	0.00	0.10	0.66	1.0
<i>Clostridium</i> spp.	−0.08	−0.25	0.69	1.3
LAB on MRS	−0.13	0.87	−0.30	1.3
LAB on M17	−0.21	0.84	−0.28	1.4
Proportion Variance	0.26	0.24	0.12	-
Cumulative Variance	0.26	0.51	0.63	-

The first component (PC1), which explains 26% of the data variability, presents a highly positive correlation to a_w ($R = 0.87$) and moisture ($R = 0.84$) and a highly negative association with pH ($R = -0.85$) (Table 1). For this reason, PC1 explains that slower drying is required for efficient fermentation. Moisture and water activity (a_w) are measurements related to the availability of water in foods, with the first pertaining to total water in a food, both in free and bound forms, while a_w is an expression of water available in a food in its free form only. This free water is essential to bacterial biological functions, and a rapid reduction in its levels results in an abrupt drop in microbial growth. In fermented foods, this hinders the development of essential fermentative bacterial communities, contributing to a higher pH of the product and a rise in spoilage and pathogenic bacterial populations [12]. In accordance with the quality maps depicted in Figure 1, the producers “Mirandela1” and “VilaFlor” apply slower/longer drying in their production process, while producers “Braganza2”, “MirandaDoDouro”, “Vimioso” and “Vinhais3” seem to employ a quicker drying period.

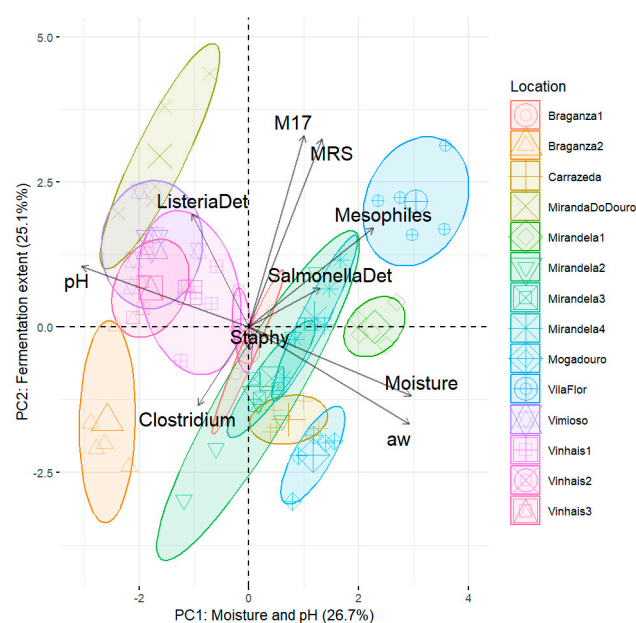


Figure 1. Quality maps of the principal components one and two of the physicochemical and microbiological attributes of “chouriço de carne” sausage, showing projections of the individual samples from each of the fourteen producers and variable loadings.

As for the second component (PC2), explaining 24% of data variation, it is highly and positively correlated with LAB (isolated from MRS, $R = 0.87$; and from M17, $R = 0.84$) and mesophilic bacteria ($R = 0.79$) and characterizes the extent of fermentation or the length of production (Table 1). Longer fermentation/maturation times are necessary for a sustained increase in mesophilic lactic acid bacteria. In turn, greater LAB populations should lead to faster and stronger pH drop, which is required for microbial safety, and LAB metabolic by-products also contribute to flavor development in the finished product [12]. The quality maps (Figure 1) show that sausages from the producers “MirandaDoDouro” and “VilaFlor” went through a longer or more efficient fermentation, while the producers “Mogadouro”, “Carrazeda” and “Braganza2” employed a shorter fermentation period in the production of their sausages, or their fermentation was less successful.

The third component (PC3), although having a moderate weight (12% of the data variability), is positively associated with *S. aureus* ($R = 0.66$) and *Clostridium* spp. ($R = 0.69$), describing sausages of low microbiological safety (Table 1 and Figure 2). *S. aureus* and *Clostridium* spp. were detected in ten and six factories each and simultaneously in five (“Braganza2”, “Mirandela2”, “MirandaDoDouro”, “Vinhais1” and “Vinhais2”). Knowing that *S. aureus* is a normal commensal bacterium known to asymptotically colonize the human skin and mucous membranes, and *C. perfringens* is found widely in environmental soil as well as in the intestinal tract of humans and animals, and adding to the fact that contamination of foods generally occurs due to poor hygiene of food handlers and/or high initial loadings in raw materials, it is clear to see a link between poor manufacturing practices and poor hygiene conditions [12].

The occurrence of *Salmonella* spp. or *Listeria* spp. was not correlated with any of the three principal components as these variables had no significant loading.

The detection of such pathogenic agents in these meat products emphasizes the urgent need to reinforce food safety knowledge and practices among Portuguese artisanal producers.

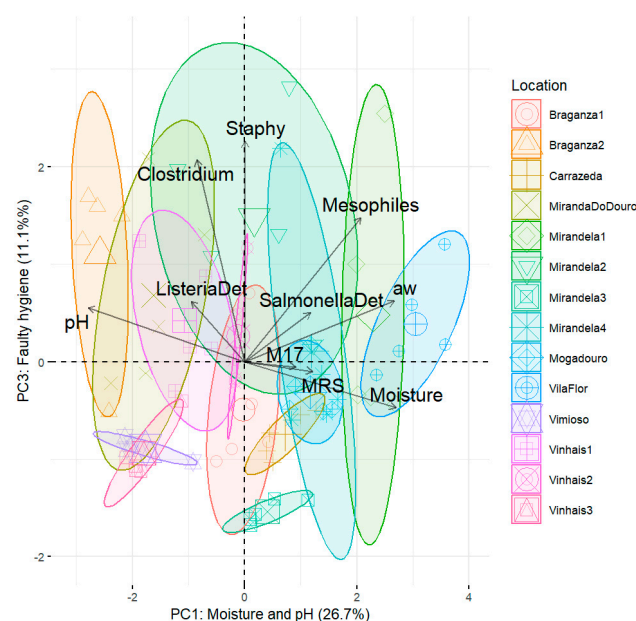


Figure 2. Quality maps of the principal components one and three of the physicochemical and microbiological attributes of “chouriço de carne” sausage, showing projections of the individual samples from each of the fourteen producers and variable loadings.

4. Conclusions

This study has established three factors of quality that directly affect the variability of chouriço produced in an artisanal manner: the duration of drying, the extent of fermentation and microbiological safety. The results showed great variability between sausages of different producers within the same geographic region, and *S. aureus*, *Clostridium* spp., *Salmonella* spp. and *Listeria* spp. were identified in sausages of several producers, highlighting the need for stricter microbiological control and standardization of production processes among artisanal producers of chouriço.

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