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MICROBIOLOGICAL AND PHYSICOCHEMICAL ASSESSMENT OF ARTISANALLY PRODUCED "ALHEIRA" FERMENTED SAUSAGES IN NORTHERN PORTUGAL

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Introduction

- Alheira is a traditional non-ready-to-eat sausage produced mainly in Northern Portugal
- Traditionally made of a mix of poultry and pork, bread and seasonings
- New formulations using game meat, codfish, mushrooms or even vegetarian/vegan options are also available in the market

Figure 1. Ingredients used in alheira formulation

Alheira production

- Cooked meats are shredded and mixed with salt, garlic, spices and sliced bread soaked in hot broth, to form a non-uniform paste
- This paste is stuffed into natural casings, and left to dry and mature at cold temperatures for 7-14 days

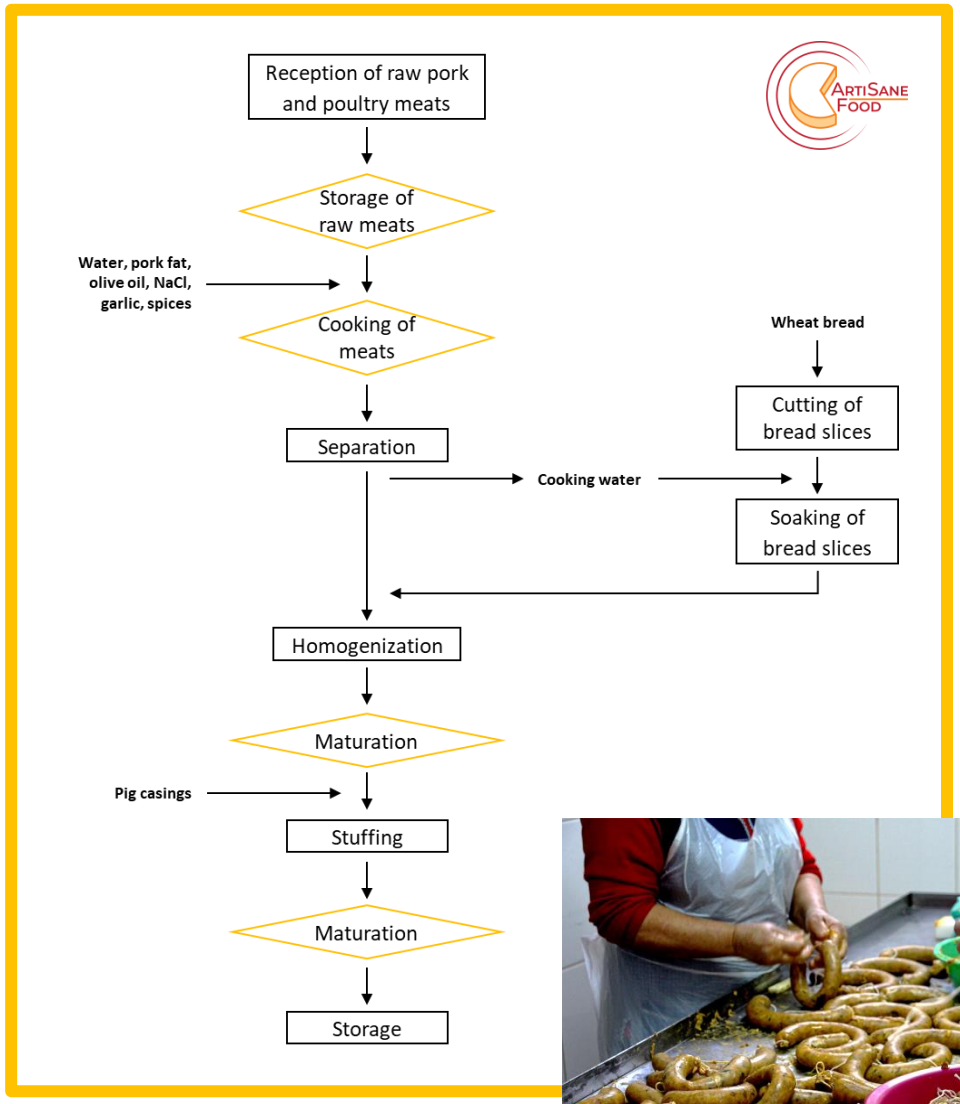
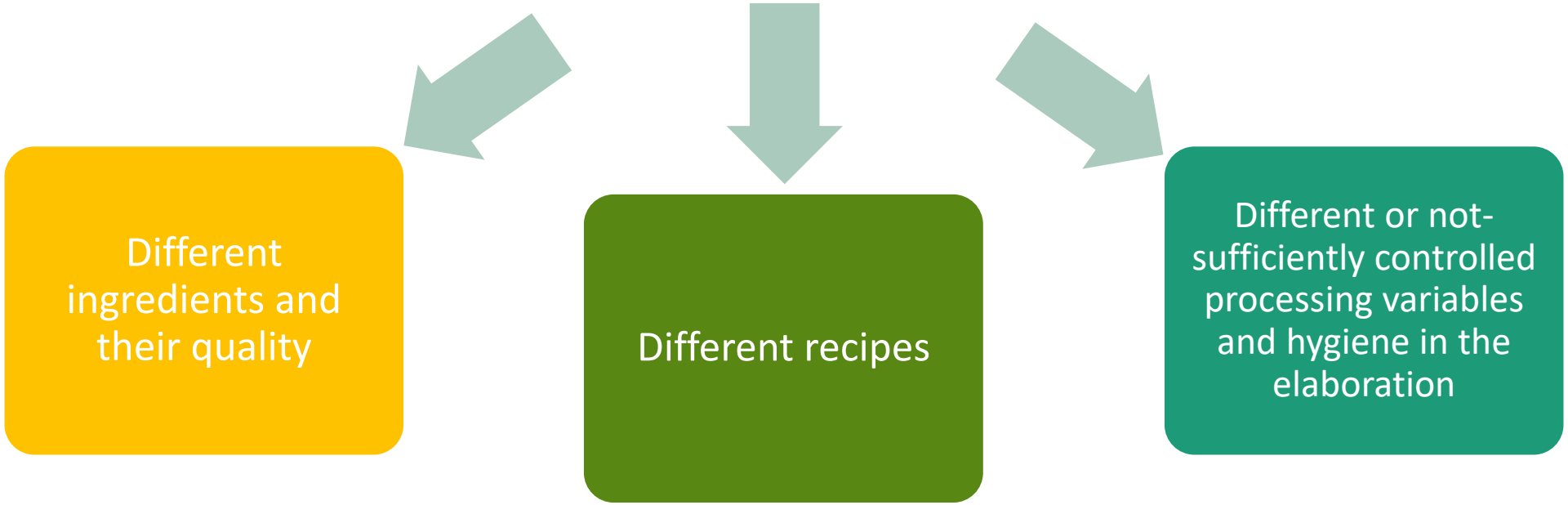


Figure 2. Alheira production fluxogram

Figure 3. Alheira stuffing process

Quality of artisanal alheira

- Quality characteristics of alheira (physicochemical, nutritional, microbiological and sensorial attributes) are highly variable between regional producers, but also between batches of production of the same enterprise



Objectives

i. To evaluate the variability in relevant physicochemical and microbiological attributes of alheira sausages elaborated by representative artisanal producers of Northern Portugal

ii. To understand the associations between these attributes through the derivation of 3D quality maps based on principal component analysis

Materials and methods

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40 alheiras

5 alheira sausages from the same production batch

8 different artisanal establishments

Vila Real
Bragança
Chaves
Mirandela
Mogadouro
Miranda do Douro
Vimioso
Valpaços

Figure 4. Districts of Portugal. The region of Trás-os-Montes appears in yellow.

- Purchased 1-2 days after production and subjected to physicochemical and microbiological analysis within 24 hours after purchased
- Casings were carefully removed from the sausages under aseptic conditions, and the contents were divided for physicochemical and microbiological analyses

Materials and methods

- Physicochemical analysis

  - **pH**
  - **Water activity (a_w)**
  - **Moisture content**
  - **Ashes content**
  - **Protein content**

- Microbiological analysis

  - **Total mesophiles**
  - **Lactic acid bacteria** (LAB in MRS and M17)
  - ***Staphylococcus aureus***
  - **Presumptive *Clostridium perfringens***
  - **Presence of *Salmonella* spp.**

Materials and methods

- Statistical analysis
  - Data from the 11 attributes were subjected to a principal component analysis (PCA), to summarize the information provided by the physicochemical and microbiological characteristics as well as their interrelationships
  - From the 3D-PCA, maps of physicochemical and microbiological quality were built from the projection of sample scores onto the span of the principal components
  - Scores were clustered by artisanal producer (i.e., location; not disclosed in the present study)

Results and discussion

- The **physicochemical and microbiological quality** of alheira sausages presented **considerable variability**

Tables 1-2. Producer-specific mean values for physicochemical and microbiological analysis

Physicochemical analysis	
pH	4.034 – 4.606
a_w	0.9758 – 0.9969
Moisture content	45.39 – 58.36%
Protein content	17.78 – 28.00% db
Ash content	2.79 – 4.71% db

Microbiological analysis	
Mesophilic counts	7.161 – 9.679 log CFU/g
LAB counts	7.704 – 11.00 log CFU/g
S. aureus counts	1.699 – 6.021 log CFU/g
Presumptive *C. perfringens*	<0.699 – 1.699 log CFU/g

- ***Salmonella* spp. was detected in 4 of the 8 sampled artisanal producers** at an incidence of 0.20 (one positive sample out of the five samples tested)

Results and discussion

Foods 2020

- **Three meaningful components** were extracted from the PCA, accounting for **63% of data variability**

Variable	PC1	PC2	PC3	Communalities
pH	-0.23	0.35	**0.86**	1.5
a_w	0.24	**-0.84**	0.17	1.2
Moisture	-0.05	**-0.73**	-0.04	1.0
Ashes	-0.48	0.47	0.05	2.0
Protein	0.11	-0.20	**0.78**	1.2
Total mesophiles	**0.85**	-0.14	-0.26	1.2
Staphylococcus aureus	0.58	0.47	0.37	2.7
Clostridium spp.	0.25	**0.70**	0.11	1.3
LAB on MRS	**0.79**	-0.05	0.12	1.1
LAB on M17	**0.81**	0.14	-0.34	1.4
Salmonella spp.	0.35	0.04	0.10	1.2
Proportion Variance	0.26	0.21	0.16	-
Cumulative Variance	**0.26**	**0.48**	**0.63**	-

Table 3. Coefficients of correlation of the physicochemical and microbiological characteristics with the three Varimax-rotated factors (PC1, PC2, PC3) along with communalities and explained variances

Results and discussion

- **The first component (PC1)** explained **26% of data variability**
- **Highly correlated with LAB** (on MRS agar (R=0.79); on M17 agar (R=0.81)) **and mesophiles** (R=0.85) and more **weakly correlated with *S. aureus*** (R=0.58)
- PC1 was labelled ***longer processing duration***, as longer fermentation times (or more efficient fermentations) tend to produce greater populations of mesophiles and LAB
- If *S. aureus* contaminates the alheira mixture, its survival depends on an insufficient drop in pH during the first stage of fermentation. It would explain the weaker correlation of *S. aureus* with PC1, since in some cases this pathogen can either increase or decrease during processing.

Variable	PC1
pH	-0.23
a_w	0.24
Moisture	-0.05
Ashes	-0.48
Protein	0.11
Total mesophiles	**0.85**
Staphylococcus aureus	0.58
Clostridium spp.	0.25
LAB on MRS	**0.79**
LAB on M17	**0.81**
Salmonella spp.	0.35
Proportion Variance	0.26
Cumulative Variance	**0.26**

Results and discussion

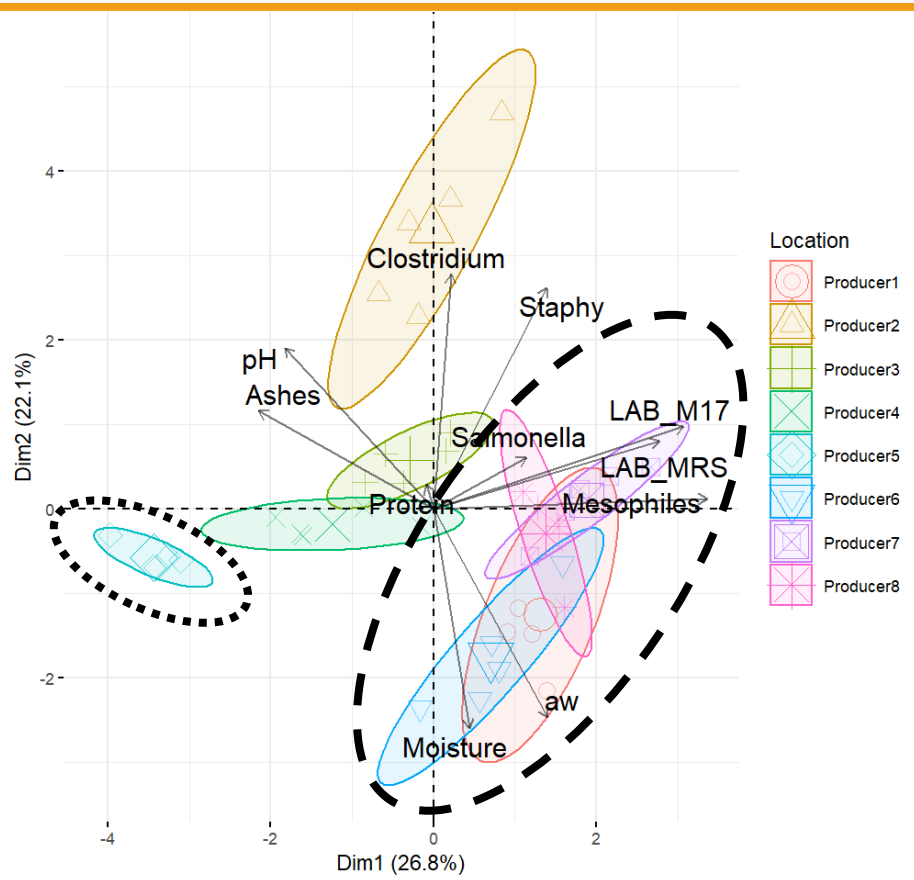

Figure 5. Map of the first and second principal component of the physicochemical and microbiological characteristics of alheira sausage with projections of samples from eight artisanal producers of Northern Portugal

- **Producers 1, 6, 7 and 8** seem to employ a **longer processing time** for the production of alheira sausages, **or** have a **more efficient fermentation process**
- **Producer 5** appears to have the **shortest alheira production time**, **or** has **delayed fermentation**

Results and discussion

- **PC2** (21% of total variability) was **highly and inversely correlated with moisture** (R=-0.73) **and a_w** (R=-0.84), and **directly correlated with presumptive *C. perfringens* counts** (R=0.70)
- The **inverse correlations imply** that **drier alheiras tended to present higher counts of *C. perfringens***
- PC2 was labelled as ***greater dehydration***
- Greater dehydration of alheira sausages can arise from longer drying times or higher drying temperatures

Variable	PC2
pH	0.35
a_w	**-0.84**
Moisture	**-0.73**
Ashes	0.47
Protein	-0.20
Total mesophiles	-0.14
Staphylococcus aureus	0.47
Clostridium spp.	**0.70**
LAB on MRS	-0.05
LAB on M17	0.14
Salmonella spp.	0.04
Proportion Variance	0.21
Cumulative Variance	**0.48**

Results and discussion

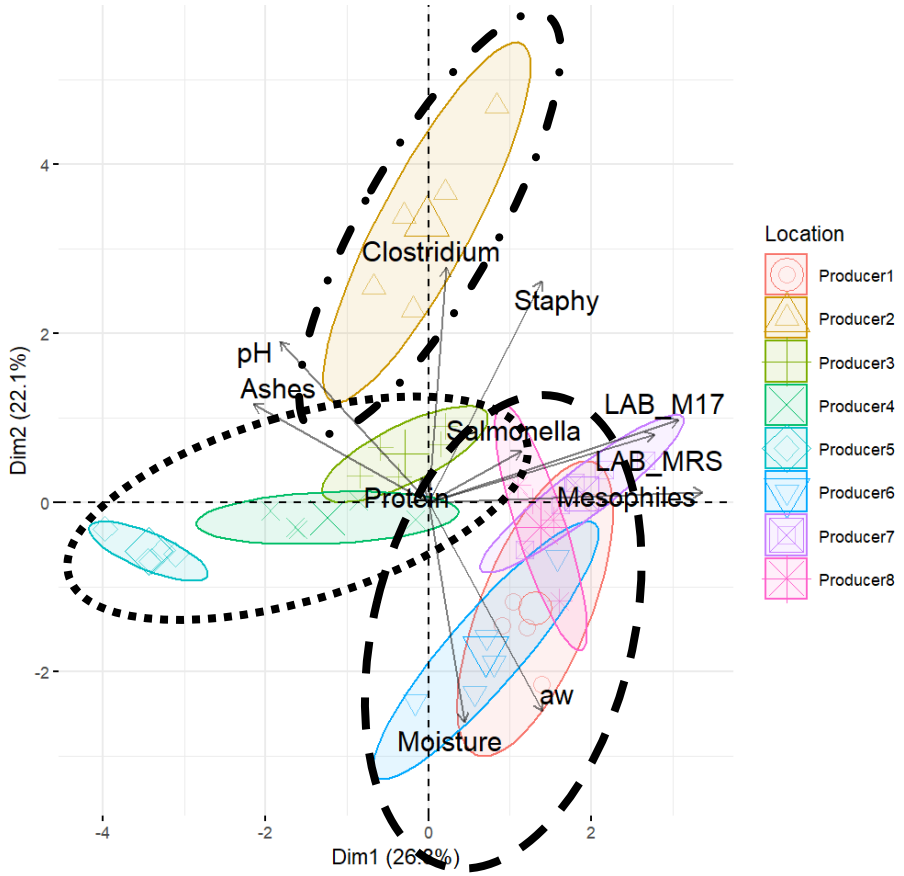

Figure 5. Map of the first and second principal component of the physicochemical and microbiological characteristics of alheira sausage with projections of samples from eight artisanal producers of Northern Portugal

- **Producers 1, 6 and 8** produced sausages with **overall higher moisture content, yet, of variable moisture** (i.e., larger ellipses along PC2 axis)
- **Producers 3, 4 and 5** elaborated **drier sausages of more consistent moisture content** (i.e., smaller ellipses along PC2)
- **Producer 2** elaborated **the most dehydrated sausages**, although their drying process may be not fully controlled (i.e., large ellipse along PC1 axis)

Results and discussion

- The third component (**PC3**) explained 16% of the data variability, and is **highly correlated** with only two variables, **pH** (R=0.86) **and protein content** (R=0.78)
- Since in alheiras the main source of protein is the meat, PC3 can be labelled as ***higher meat proportion in the formulation***
- The probable tendency of artisanal producers to use pig meat of high pH (i.e., DFD meats) may explain why protein content and pH of alheiras seem so highly associated
- Another explanation is that formulations with a lower proportion of meat are compensated with a higher proportion of regional bread, a foodstuff of lower pH. Thus, batters of higher proportion of meat will tend to have higher pH

Variable	PC3
pH	**0.86**
a_w	0.17
Moisture	-0.04
Ashes	0.05
Protein	**0.78**
Total mesophiles	-0.26
Staphylococcus aureus	0.37
Clostridium spp.	0.11
LAB on MRS	0.12
LAB on M17	-0.34
Salmonella spp.	0.10
Proportion Variance	0.16
Cumulative Variance	**0.63**

Results and discussion

- **Producers 1, 4 and 7** employed a **higher concentration of meat in their formulations**

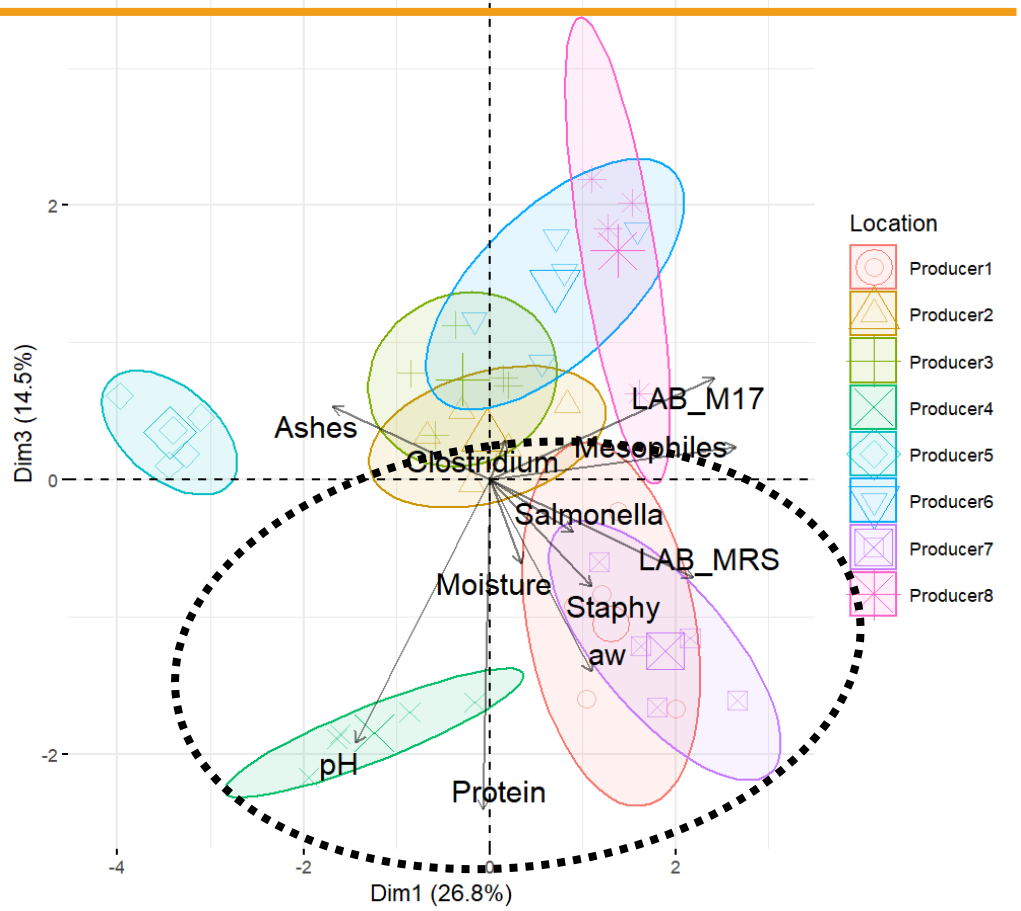

Figure 6. Map of the first and third principal component of the physicochemical and microbiological characteristics of alheira sausage, with projections of samples from eight artisanal producers of Northern Portugal.

Conclusions

- This work identified three quality axes supporting the variability in artisanal alheiras:
  - ***duration of fermentation***
  - ***extent of dehydration***
  - ***proportion of meat in formulation***

- It has also highlighted the need to implement **better microbiological control** and **process standardization** during the production of artisanal alheiras

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Thank you for your attention!