Research article

Fat content reduction and lipid profile improvement in Portuguese fermented sausages *alheira*

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A B S T R A C T

Due to the current trend to reduce fat consumption, the meat food industry, has been increasing the strategies to produce and commercialize products where the reduction or even elimination of saturated fat is an important goal. This study aimed to test different formulas to reduce the fat content and improve the lipid profile in the Portuguese fermented sausage *alheira*. Data indicate that the three composition formulas of *alheiras* tested are not a hypercaloric product and the fatty acid profile could be improved changing the sources of fat and specie of meat, particularly reducing the palmitic and stearic acids and the myristic acid contents and increasing the oleic, linoleic and palmitoleic acid contents. The industry could diversify its offer with two new composition formulas, a pork-free *alheira,* and oil-free *alheira,* reducing the fat content and improving the fatty acid profile.

1. Introduction

The meat food industry has been increasing the strategies to produce and commercialize products where the reduction or even elimination of saturated fat is an important goal. One of the most important challenges of the processed meat industry is finding solutions to produce high nutritional and healthier products maintaining the flavor traditions and consumer expectations and acceptability. The original *alheira* designated as "the sausage of the Jews," was a horseshoe-shaped sausage without pork meat, made by the Portuguese Jews during the period of the Inquisition (late 16th and 17th centuries) to distinguish themselves as "new Christians." The popularity of this fermented sausage formerly composed mainly of poultry, bread, olive oil, garlic, and pepper grew over time and today mixes up all kinds of meats, especially fat pork. This sausage has been the subject of several studies of physicochemical and sensory characteristics (Patarata et al., 2008) processing conditions (Ramalhosa et al., 2012; Campos et al., 2013; Gomes et al., 2013) microbiological characterization and safety (Ferreira et al., 2006, 2007; Albano et al., 2008) or even the microbiological and chemical characterization of a non-meat based *alheiras* (Azevedo et al., 2020).

Due to the trend to reduce fat consumption, the meat food industry has been increasing the strategies to produce and commercialize products where the reduction or even elimination of saturated fat is an important goal. One of the most crucial challenge of the processed meat

industry is finding solutions to produce high nutritional and healthier products maintaining the flavor traditions and consumer expectations and acceptability. This study aimed to test different formulas to reduce the fat content and improve the lipid profile in the Portuguese fermented sausage *alheira*.

2. Material and methods

There is no single formulation for this sausage called *alheira*. The composition can vary according to the type and certified brand with Protected Geographical indication (PGI). The PGI is acquiring designations according to the type of meat that composes it since they all have thinly sliced bread (wheat bread with very little yeast and low salt content, so that there is no yeast inside the sausage), broth meat (to get the bread to absorb as much broth as possible), garlic, paprika, salt, and olive oil.

2.1. Alheiras manufacturing and sampling

The Bísaro Salsicharia Industry that usually produces four different types of *alheira* to meet the most demanding consumers tested 3 new formulations, changing the type of meat (with and without pork), reducing and changing the fat content (pork fat and without oil). The pork back fat as well the pork belly were from the Bísaro pig breed. The

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boiled meat is carefully boned, chopped with a knife or shredded, mixed with the other ingredients, but in such a way that it is felt by the consumer when the *alheira* is tasted. All the ingredients are mixed, and the dough is stuffed into a casing (pork, cattle, or synthetic) and dried slightly smoked and fermented naturally. Table 1 shows the composition of the three types of *alheiras* manufactured. Two replications of the three types of *alheiras* were manufactured at different times. For each replicated lot, three samples of each *alheira* type were randomly selected, and each sample was analyzed in triplicate for each physicochemical analysis.

2.2. Physicochemical analysis

The pH measurement was determined according to the Portuguese standard NP 3441 (2008) using a Crison 507 pH-meter equipped with a 52–32 puncture electrode. Water activity was assessed according to AOAC (1990) using a probe HigroPalmAw1 rotronic 8303, Bassersdorf, Switzerland. Hydroxyproline determination of collagen content and concentration, protein, ashes and mointure were assessed according to the Portuguese standards NP 1987 (2002), NP 1612 (2002), NP 1615 (2002) and NP 1614 (2009), respectively. Total chloride content determined by the method specified in Portuguese standard NP 1845 (1982) expressed as sodium chloride as a percentage by mass.

A *Trás-os-Montes* Origin Protected designation (DOP) olive oil was used with the following fatty acids profile: 11.2% C16:0, 0.2% C17:1, 3.3% C18:0, 75.2% C18:1n-9, 7.7% C18:2n-6, 0.4% C20:0, 0.8% C18:3n-3, 0.2% C20:1n-9, 0.1% C22:0 (Teixeira, 2015). The fatty acids profile of Bísaro pork belly is:1.3% C14:0, 22.3% C16:0, 2.1% C16:1, 11.9% C18:0, 41.9% C18:1n-9, 15.7% C18:2n-6, 1.2% C18:3n-3, 0.78% C20:1n-9 (Teixeira et al., 2019).

2.3. Fatty acid composition analysis

The total amount of lipids was extracted from 25 g of the sausage sample, according to the procedure by Folch et al. (1957). Fifty milligrams of fat were used to determine the fatty acid profile. The fatty acids were transesterified according to the method described by Shehata et al. (1970) with the modifications of Domínguez et al. (2015); 4 mL of a sodium methoxide solution were added, vortexed every 5 min for 20 min at room temperature, then 4 mL of a H_2SO_4 solution (in methanol at 50%), vortexed briefly and vortexed once more before adding 2 mL of distilled water. The organic phase (with the methyl esters of fatty acids) was extracted with 2.5 mL of hexane. The FAMEs separation and quantification were performed using a gas chromatograph (GC-Agilent 6890N; Agilent Technologies Spain, S.L., Madrid, Spain) provided with a flame ionization detector and an automatic sample injector HP 7683, and a Supelco SPTM-2560 fused silica capillary column (100 m, 0.25 mm i. d., 0.2 μm film thickness). The chromatographic requirements were: initial temperature of the column was 120 °C, for 5 min, fixed out to rise at a rate of 5 °C·min1 up to 200 °C, maintaining this temperature for 2 min, then at 1 °C·min1 up to 230 °C for 3 min. The dertector and injector were held at 280 and 260 °C, respectively. Helium was used as the carrier gas at a fixed rate flow of 1.1 mL·min1, with the column head pressure set at 35.56 psi. The separation rate was 1:50, and 1 μL of the solution was injected. Previously the methylation was added to the samples the non-adecanoic acid (C19:0) at 0.3 mg·mL1 as internal standard. Individual FAMEs were identified by comparing their retention times with those of authenticated standards (Supelco 37 component FAME Mix). Data were expressed in g/100 g of fatty acid.

To acess the lipid quality the index of atherogenicity (IA) and the index of thrombogenicity (IT) were used according Ulbricht and Southgate (1991):

1. AI = (C12:0 + 4 x C14:0 + C16:0)/($\sum$MUFA + $\sum$PUFA);
2. TI = [(C14:0 + C16:0 + C18:0)/(0.5 x $\sum$MUFA +0.5 x $\sum$PUFAn6 + 3 x $\sum$PUFAn3 + (PUFAn3/PUFAn6))].

2.4. Statistical analysis

A Standard Least Square model was fitted to analyze the differences between the three types of *alheira*. Data were analyzed using the statistical package JMP® Pro 13.1.0 by Copyright © 2016 SAS Institute Inc. The predicted means obtained were ranked based on pair-wise least significance differences and compared using the Tukey's HSD test for *P < 0.05, **P < 0.01 or ***P < 0.001 significance levels.

3. Results and discussion

The results of the physicochemical analyzes are shown in Table 2. Statistically significant differences were observed for pH, which varied between 6.0 to 5.4 for *alheiras* type 1 and type 3, respectively. The pH values found are in line with those presented in previous studies by Ferreira et al. (2006), Patarata et al. (2008), or Azevedo et al. (2020). The a_w value was also different between the three types of *alheiras* as well as the moisture, with higher values for *alheiras* type 3 and values that are within the expected considering the values presented by Patarata et al. (2008) and Ferreira et al. (2006) and Campos et al. (2013) or Barros et al. (2018). The high moisture values verified are characteristic of this product because it is a sausage of boiled meats that mixed with bread are then soaked with the meat cooking broth. The salt content expressed as % of sodium chloride varied between 1.6 and 1.9% and is within the values indicated by several studies with *alheiras* (Ferreira et al., 2006; Patarata et al., 2008; Campos et al., 2013). The value of the percentage of protein was higher in type 2 sausages, with about 12% compared to other types that are around 10%. The considerable variability in the different formulations of sausages available on the market means that the values of the present study are close to the products analyzed by Patarata et al. (2008) and Ferreira et al. (2006) higher than the 8.3% recorded by Marcos et al. (2016) but lower than the 21.2% found by Campos for Raw

Table 1. Composition of the three types of *alheiras*.

Component	Alheira type		
	Alheira 1	Alheira 2	Alheira 3
Bread	40 kg	40 kg	40 kg
Garlic	400 g	400 g	400 g
Salt	1.5 kg	1.5 kg	1.5 kg
Paprika	400 g	400 g	400 g
Olive oil	5 L	2 L	NO
Pork back fat	No	30 kg	30 kg
Pork belly lean meat	No	No	30 kg
Partridge and duck meat	50 kg	No	No
Duck, partridge, and chicken	NO	50 kg	No
Broth meat	110 L	110 L	110 L

Table 2. Predicted values (mean ± standard error) for physicochemical composition.

| | Alheira type | | | P-values |
	Alheira 1	Alheira 2	Alheira 3	
a_w	0.940 ± 0.001$_a$	0.940 ± 0.001$_a$	0.960 ± 0.001$_b$	***
pH	6.00 ± 0.02$_a$	5.70 ± 0.02$_b$	5.40 ± 0.02$_c$	***
Chlorides (%)	1.80 ± 0.05$_{ab}$	1.90 ± 0.05$_a$	1.60 ± 0.05$_b$	**
Ash (%)	2.9 ± 0.4	2.7 ± 0.4	2.1 ± 0.4	ns
Moisture (%)	55.5 ± 0.6$_a$	57.9 ± 0.6$_b$	60.7 ± 0.6$_c$	***
Protein (%)	10.0 ± 0.5$_b$	12.0 ± 0.5$_a$	9.9 ± 0.5$_b$	**
Total fat (%)	13.6 ± 0.7$_a$	7.6 ± 0.7$_b$	8.6 ± 0.7$_b$	***

NS: not significant p > 0.05; means with different letters subscript are significant for *, **, *** to significant levels p < 0.05; p < 0.01; p < 0.001, respectively.

alheiras. Regarding the percentage of fat, the *alheiras* type 2 and 3 had significantly lower values than the type 1 sausage, with a reduction of between 6 and 5% in the fat content, respectively. Also, for the amount of fat, there is significant variability between different sausages. The values of the present study are considerably lower than the 18.4% presented by Ferreira et al. (2006), the 19.5% reported by Patarata et al. (2008), or the 18,1% Marcos et al. (2016).

However, the total amount of fat is not, in itself, the quality parameter of meat products. Knowledge about the degree of saturation and the profile of fatty acids is increasingly important with an impact on quality and consumer healthy food once the unfavorable lipid profile of processed meat products is generally associated with harmful cholesterol levels and heart diseases. The fatty acid profile of the three types of *alheiras* shown in Table 3, is mainly composed of monounsaturated fat,

Table 3. Predicted values (mean ± standard error) for fatty acid profile (expressed in g/100 g of fatty acids).

| Fatty acids | Alheira type | | | P-values |
	Alheira 1	Alheira 2	Alheira 3	
C 10:0	0.05 ± 0.01$_a$	0.05 ± 0.01$_a$	0.02 ± 0.01$_b$	**
C 12:0	0.11 ± 0.02$_{ab}$	0.16 ± 0.02$_a$	0.09 ± 0.02$_b$	*
C 14:0	1.24 ± 0.08$_a$	1.07 ± 0.08$_a$	0.66 ± 0.08$_b$	**
C 14:1	0.02 ± 0.00$_a$	0.03 ± 0.00$_a$	0.06 ± 0.00$_b$	**
C 15:0	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	Ns
C 16:0	23.9 ± 0.3$_a$	22.6 ± 0.3$_b$	20.6 ± 0.3$_c$	***
C 16:1n7c	2.4 ± 0.1$_b$	2.6 ± 0.1$_{ab}$	2.9 ± 0.1$_a$	*
C 17:0	0.25 ± 0.02$_a$	0.23 ± 0.02$_a$	0.15 ± 0.02$_b$	**
C 17:1	0.26 ± 0.02$_a$	0.24 ± 0.02$_a$	0.16 ± 0.02$_b$	**
C 18:0	10.3 ± 0.6$_a$	8.9 ± 0.6$_a$	6.9 ± 0.6$_b$	**
9t-C 18:1	0.28 ± 0.01$_a$	0.24 ± 0.01$_a$	0.18 ± 0.01$_b$	***
C 18:1 n9c	46.1 ± 0.7$_b$	47.8 ± 0.7$_b$	51.8 ± 0.7$_a$	***
C 18:2n6c	12.7 ± 0.3$_b$	13.8 ± 0.3$_b$	15.0 ± 0.3$_a$	***
C18:3n6c	0.02 ± 0.00$_b$	0.04 ± 0.00$_{ab}$	0.06 ± 0.00$_a$	**
C 20:1n9	0.7 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	ns
C 18:3n3	0.55 ± 0.02$_c$	0.65 ± 0.02$_b$	0.74 ± 0.02$_a$	***
C 21:0	0.05 ± 0.00$_a$	0.05 ± 0.00$_a$	0.02 ± 0.00$_b$	**
C 20:2n6	0.40 ± 0.03$_b$	0.46 ± 0.03$_b$	0.60 ± 0.03$_a$	**
C 20:3n6	0.09 ± 0.00$_a$	0.08 ± 0.00$_{ab}$	0.07 ± 0.00$_b$	*
C 20:3n3	0.08 ± 0.00$_a$	0.07 ± 0.00$_a$	0.03 ± 0.00$_b$	**
C 20:4n6	0.29 ± 0.01b	0.35 ± 0.01a	0.31 ± 0.01ab	*
C 20:5n3	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	ns
C22:6n3	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	ns
∑SFA	36 ± 1$_a$	33 ± 1$_a$	28 ± 1$_b$	***
∑MUFA	49.8 ± 0.8$_b$	51.5 ± 0.8$_b$	55.6 ± 0.8$_a$	***
∑PUFA	14.3 ± 0.3$_c$	15.4 ± 0.3$_b$	16.5 ± 0.3$_a$	***
PUFA/SFA	0.40 ± 0.03$_b$	0.46 ± 0.03$_b$	0.60 ± 0.03$_a$	**
PUFA-n3	0.67 ± 0.01$_c$	0.76 ± 0.01$_b$	0.82 ± 0.01$_a$	***
PUFA-n6	13.5 ± 0.3$_b$	14.7 ± 0.3$_b$	16.1 ± 0.3$_a$	***
PUFA-n6/n3	20.1 ± 0.1$_a$	19.3 ± 0.1$_b$	19.6 ± 0.1$_{ab}$	**
∑trans	0.28 ± 0.01$_a$	0.24 ± 0.01$_a$	0.18 ± 0.01$_b$	***
IA index	0.45 ± 0.01$_a$	0.40 ± 0.01$_a$	0.33 ± 0.01$_b$	***
IT index	1.05 ± 0.03$_a$	0.92 ± 0.03$_a$	0.72 ± 0.03$_b$	***

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; IA index – index of atherogenicity; IT – index of thrombogenicity; NS: not significant p > 0.05; means with different letters subscript are significant for *, **, *** to significant levels p < 0.05; p < 0.01; p < 0.001, respectively.

particularly the *alheiras* type 3, with a significantly higher value of 55,6% as well as a higher level of polyunsaturated fat of 16,5%. The saturated fat in *alheiras* type 3 was between 6 – 8 % lower than *alheiras* type 2 and 1, respectively.

The results are globally in accordance with the fatty acid profile of raw *alheiras*, recorded by Campos et al. (2013) except for *alheiras* type 3 that had an exceptionally high percentage of mono and polyunsaturated fat from type 3 sausages. The results are globally in accordance with the fatty acid profile of raw sausages, recorded by Campos et al. (2013) except for *alheiras* type 3 that had an exceptionally high percentage of mono and polyunsaturated fat from type 3 sausages, 72.1% compared to 66.9 and 64.1% for *alheiras* type 2 and 1 respectively. It was also the same when compared with the values of unsaturated fat reported for several Portuguese alheiras by Marcos et al. (2016). The predominant fatty acids in decreasing order are: oleic (C18:1n-9); palmitic (C16:0); linoleic (C18:2n-6); stearic (C18:0) and palmitoleic (C16:1 n-7) and *alheiras* type 3 (without olive oil as fat source) have a significantly higher content of oleic and linoleic and significant lower content of palmitic and stearic.

The fatty acid composition indicates that the primary fat sources of *alheira* are pork fat and olive oil. The *alheiras* type 1 presented the highest saturated fat content and in terms of its composition (without pork meat or fat, the only fat source was olive oil) correspond to the original *alheira*, a pork-free sausage which appears been created by Portuguese Jews to disguise themselves as "New Christians." The meat cooking broth incorporated in the mixture of type 1 sausages, as it contains a high amount of poultry meat, particularly whole carcasses of duck and chicken, must have contributed to a higher saturated fat content compared to sausages type 2 and 3. Also the differences in the fatty acid profile between the three types of alheiras are justified by the fatty acid profiles of the oil compared to the pork belly. All three types studied have less saturated fat content and more unsaturated fat content than the traditional *alheiras* studies by Campos et al. (2013) or Marcos et al. (2016). The addition of pork meat and fat as the only fat source in the sausages 3 modified the fatty acid profile leading to a decrease of percentages of C16:0, C18:0 and C14:0 and an increase in C18:1n9 and C18.2n6 the predominant fatty acids in pork backfat (Lorenzo et al., 2012). Similar results were found by Leite et al. (2015) in a study on the effect of different pork fat levels on the fatty acid profile of sheep and goat sausages as well as in sheep and goat pâtés with different levels of pork back fat or olive oil (Teixeira et al., 2019). The myristic acid (C14:0) associated with an unfavorable lipid profile and low plasma HDL cholesterol levels (Noto et al., 2016) is purely represented in the three types of *alheiras* with only 0.66–1.24%. *Trans* fatty acids (TFA) are produced by industrial hydrogenation or by biohydrogenation in the rumens of cows and sheep (Brouwer et al., 2016) and have a potential effect on LDL-cholesterol (Wood et al., 2008). The elaidic acid (9t-C18:1) as well other monounsaturated TFA decreased HDL cholesterol concentrations and increased those of LDL cholesterol. Its consumption has a strong relationship with cardiovascular disease (CVD) Mensink (2005), and the population nutrient intake goal for TFA recommended by joint WHO/FAO expert consultation is less than 1% of total energy intake (https://www.sciencedirect.com/science/article/pii/S2405844019357792, Brouwer, 2016). The three types of *alheira* only have between 0.16 and 0.26% of TFA, less than the WHO/FAO recommendation. The nutritional quality defined through AI and TI indices varied between 0.33 - 0.45 and 0.72–1.05 respectively. Alheiras type 3 had significantly lower valus for both indices. The values found are slightly higher than those reported by Moussa et al. (2014) for salmon and sardines, but lower than those reported for tuna and mackerel except for the latter's IT value, which is considerably low (0.17). But when compared to values in turkey meat (Mauric et al., 2016) or in pig fat and muscle (Grela et al., 2014), the ones we found are within the limits of 0.30–0.50 for AI and 0.75–1.2 IT referred by the mentioned authors. When compared with the values reported by Franco et al. (2020) for fermented sausages in which the pig fat was partially replaced by linseed oil oleogels, the values in the present work are slightly higher than 0.26–0.39 for AI or 0.28–0.70 for TI.

However, in relation to the control, the values found by us are within the limits mentioned by the authors. The PUFA/SFA ratio is typically used as an indicator of the nutritional quality evaluation of the human diet. The mean ratio of PUFA/SFA recommended by the British Department of Health is more than 0.45, and WHO/FAO experts have reported guidelines for a "balanced diet" in which the suggested ratio of PUFA/SFA is above 0.4 (Wood et al., 2008). The PUFA/SFA recorded ranged from 0.6 to 0.4 for *alheiras* type 3 and type 1, respectively, and are similar to the results found by Campos et al. (2013) for *raw alheiras*. Beyond the total dietary fat intake, the n6/n3 PUFA ratio is another indicator of the quality in the diet Li et al. (2019). Lipid metabolism, inflammation, oxidative stress, and endothelial function play essential roles in the pathogenesis CVD, which may be affected by an imbalance in the n-6/n-3 PUFA ratio (Yang et al., 2015). According to Simopoulos (2004), the ratio of n-6 to n-3 PUFA should be less than 4, and the recorded values for us for the three types of *alheiras* were considerably high ratio, although within the values pointed out by Campos et al. (2013) for *raw alheiras*. Yang et al. (2015) state that the 10: 1 ratio corresponds to the average human diet, and the 5: 1 ratio is close to the commonly recommended n-6/n-3 PUFA ratio. In fact, WHO (2009), based on scientific evidence and conceptual limitations, report that there are no convincing scientific rational recommendations for a specific ratio of n-6 to n-3 PUFA.

4. Conclusions

Data indicate that these types of *alheiras* are not a hypercaloric product, and the fatty acid profile could be improved, changing the sources of fat and specie of meat, particularly reducing the palmitic and stearic acids and the myristic acid contents and increasing the oleic, linoleic and palmitoleic acid contents. The results of this study confirm that it is possible to produce *alheiras* with a reduction of total fat content in comparison with the commonly commercialized brands in the market. *Alheiras* type 1, without pork fat meat, would be an attractive solution to satisfy the growing consumer market for kosher and halal products and the *alheiras* type 3 for consumers looking for products with reduced fat content and an improving fatty acid profile. In conclusion, the industry will be able to diversify its offer with two new references, one pork-free *alheira,* and another olive oil-free *alheira*, with reduced fat content and with a more favorable fatty acid profile concerning the products produced so far.

Declarations

Author contribution statement

Alfredo Teixeira: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Alberto Fernandes: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Etelvina Pereira: Analyzed and interpreted the data.

Competing interest statement

The authors declare the following conflict of interests: Alberto Fernandes; [is an employee of Bísaro Salsicharia. This company produced the 3 new alheira sausage formulations used in our work.]

Additional information

No additional information is available for this paper.

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