CHEMICAL CHANGES IN THE LIPID FRACTION OF TRADITIONAL DRY FERMENTED SAUSAGE "PAINHO DE PORTALEGRE" DURING RIPENING

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Background

In manufacture of dry fermented sausages, the drying/ripening stage is of decisive importance to the final product sensory properties. During this process, the occurrence of complex physicochemical, biochemical and microbial phenomena affect proteins and lipids, modifying their structural integrity and physical characteristics (Gray & Pearson, 1984; Melgar *et al*., 1990). Different formulations are adopted according to cultural traditions of the geographical regions where they are produced but generally lipids represent the major fraction. The specificity of the flavour is then greatly dependent on the intrinsic composition of raw materials and on the hydrolytic and oxidative changes operated on that substrate. This is influenced by a wide range of factors namely, the relationships of environmental temperature/relative humidity/ventilation, type of ingredients/spices and duration of ripening.

Objectives

The present study was undergone to evaluate the chemical composition of "Painho de Portalegre", a traditional dry fermented sausage, and the variations occurred during the ripening period.

Materials and methods

Preparation of Painho de Portalegre and sampling – Chilled chunks of pork lean and fat were ground to obtain mincing sizes of about 2 cm (hole plate grinder), mixed together (Sample 1) and added of paprika paste, NaCl and garlic paste. After a holding period of 3 days at 0-2˚C (sample 2), the mixture was stuffed in natural casings (rectal portion of pig intestine) and transferred to a traditional drying/smoking house (firewood burning inside) until an a_w value of 0.88-0.85 was reached. Depending on the prevalent atmospheric conditions, this period varied between 30 to 40 days. Samples 3, 4, 5, 6 and 7 were taken after 6, 15, 20, 30 and 40 days of the ripening process. After processing the product was transferred to a room temperature environment where they were stored up to day 60 and then packaged under vacuum (BB4L plastic bags) up to 180 days. During the storage period samples 8, 9 and 10 were taken at days 55, 120 and 180.

Physicochemical analysis – pH was determined using a Metrohm 654 pH meter, according to the method described in NP 3441 (1990). Water activity evaluation was carried out with Rotronic Hygrolab using a probe AwVC-DIO. Moisture was determined using the method described in NP 1614 (2002). Total lipids were extracted by the procedure of Folch *et al* (1957). Protein was determined through total nitrogen using the Kjeldahl method. Sodium chloride content was determined following the procedure described in NP 1845 (1982).

Determination of free fatty acids – Lipids were extracted from Paínho samples with chloroform/methanol (2:1) according to the method of Folch (1957). FFA were purified from neutral lipids using an anionic exchange resin (Amberlyst A-26). An aliquot of 500 mg of lipids was dissolved in 30 ml of a mixture of acetone/methanol (2/1) as described by Alasnier *et al.* (2000) and Needs. The mixture with the lipid fraction was mixed with 750 mg of the exchange resin, was added with 1 ml of the internal standard solution (20µl of heptanoic acid/ml acetone/methanol) and shaken for 60 min. The resin was washed five times with 5 ml of acetone/methanol mixture for removing of neutral lipids. After drying for 1 h at ambient temperature, the resin with adsorbed FFA was placed in a reaction vial for FFA methylation with BF_3 in methanol (14%) as described by

Partidário (1998). The methyl esters were separated and quantified by gas chromatographic analysis, on a TRACE GC, series 2000 instrument (Thermo Quest, USA), using a DB-23 (50% cyanopropyl-

methylpolysiloxane) fused silica column (60 m long, 0,25 mm i.d., 0,25µm film thickness), supplied by J&W Scientific, Folsom, USA.
For the determination of fatty acid composition of triacylglycerols, extracted lipids were dissolved in isooctane and transesterified with a methanolic solution of KOH (0,1N). Methyl esters were separated and quantified as just described.

Results and discussion

Table 1 shows the mean, standard deviation, maximum, and minimum values found out for some general parameters, which are important to the characterisation of centesimal composition of "Painho de Portalegre". For a similar water availability (mean a_w=0.87), this traditional dry fermented sausage presented some variation in protein/fat relationship among producers, from a minimum of 0.30 up to a maximum value of 0.62. Sodium chloride content, an important factor influencing the ripening process evolution, in particular the rate and extension of the lipid autoxidation process, also showed variations of about 2%, between 3% and 5% approximately. The continuing decrease of pH, which likely affects the preservation ability, the physical aspect and the taste characteristics of the product, is closely associated to microbial development along the processing stages. The degree of variation verified in this study is enough expressive to differentiate the final products into acid (pH< 5.0) or moderately acid (pH>5.5).

Table 1 – Physico-chemical parameters of "Painho de Portalegre" composition.

	Protein (%)	**Moisture (%)**	**Fat (%)**	**pH**	**aW**	**NaCl (%)**	**Acidity** (ml NaOH 0,1N)
Mean	20.44	31.12	43.45	5.26	0.87	3.95	4.34
SD	2.77	3.52	4.63	0.31	0.02	0.56	0.39
Min	16.00	25.14	38.15	4.74	0.85	3.15	3.79
Max	23.80	37.01	51.85	5.61	0.89	4.90	4.73

Results for the fatty acids composition of pork fat used in "Painho de Portalegre" production showed a saturated/unsaturated fatty acids relationship lower than the usual profile referred for this type of product (Ordónez *et al*., 1999) (Table 2). This difference (0.48 vs 0.69) is basically due to the lower presence of C16 (-4.2%) and C18 (-4.6%) elements and a significantly higher concentration of oleic acid (+9.4%) and could be ascribable to the rearing and feeding procedures applied to the animal production system.
Raw material analysis showed already important amounts of FFA, reaching about 707 mg/100g of fat (Table 2). Among these compounds, C18:1 (9), C16 and C18 predominated, with values of 276.5 mg, 144.7 mg and 112.9 mg/100g of fat, respectively, keeping that order all over the ripening process. Linoleic acid appeared in 4th place with 80.6 mg/100g fat, and was the only detected polyunsaturated fatty acid. All the other elements appeared at much lower concentrations. At this early processing stage, MUFA slightly predominate over SFA, making a SFA/MUFA relationship of 0.93. During the standing of spiced raw materials in the refrigerator (flavour up taking and natural flora selection purposes) FFA formation was just slightly increased (+5.7%). However, a remarkable change occurred in the FFA profile, due a the significant rise in SFA (497.5 mg vs 301.9 mg) and, on the contrary, a sound reduction in MUFA and PUFA concentration, approximately –40% in both classes. As a consequence, the SFA/MUFA relationship more than doubled (0.93 vs 2.44). The evolution of C16/C16:1 and C18/C18:1 quotients clearly shows that the relative extension of that saturation mechanism affected mostly the former (6x vs 1,6x). However the possible impact over the product sensory quality coming from oxidation of oleic fatty acid
should also be emphasised due to its much higher concentration in the raw material prior the chilling holding stage (303 mg- C18:1 (9)+(11) vs 18.6 mg-C16:1 (7)+(9)).

Table 2- Fatty Acid (FA) profile and Free Fatty Acid (FFA)content of "Painho de Portalegre" along the ripening phase.

	FA profile (g/100g FA)	FFA (mg/100g fat)									
		S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
C8	0.29										
C10	0.07										
C10:1	0.12										
C12	0.08	2.77	1.67	1.79	2.30	2.24	2.81	4.22	4.20	8.79	10.15
iC14		4.08	2.66		1.29		3.32	2.29	0.97		
C14	1.21	11.41	10.01	14.47	21.40	24.56	32.16	41.70	56.23	94.79	115.73
C14:1		2.30			2.23		2.99	1.12	1.36		
C15	0.05	0.63	2.48	0.95	1.21	1.96	2.46	1.75	2.77	3.23	3.53
iC16		17.74	19.86		1.24		9.53	3.84	1.96	3.13	1.70
C16	20.82	144.68	224.44	264.90	342.86	398.19	514.36	668.36	801.37	1221.38	1408.58
C16:1 t			0.90		1.09		4.12				
C16: 1 (7)	0.40	1.82	1.22	3.28	7.71	6.66	7.95	10.44	13.98	21.63	18.80
C16: 1 (9)	2.79	16.78	9.80	18.98	45.25	37.16	51.85	73.22	113.92	158.22	164.40
C17	0.31	3.10	2.91	3.46	6.73	9.61	9.75	10.69	10.75	15.72	15.33
C17:1	0.35	1.34		1.39	4.88	7.12	5.52	7.13	10.55	3.77	15.70
iC18		4.60	2.57								
C18	9.34	112.90	230.33	235.60	293.29	355.08	410.08	516.24	507.55	616.31	543.33
C18:1 t	0.36		1.05			0.82	13.57	10.11	19.24	28.40	15.74
C18: 1 (9)	47.86	276.49	168.16	333.22	758.21	658.56	887.50	1121.06	1736.59	2181.83	2180.19
C18: 1 (11)	3.30	26.53	20.37	35.81	88.26	77.84	99.95	124.35	200.69	308.44	265.41
C18:2	8.50	80.60	45.19	121.60	320.75	304.75	359.56	403.94	578.21	740.57	664.33
C18:2 isóm.	0.17						1.56		7.53	8.09	6.49
C18:3	0.79			10.04	28.05	23.44	27.55	34.06	46.78	69.96	55.16
C18:4	0.06										
C20	0.24		0.56							6.82	5.92
C20:1	0.92										
C20:1 (9)			1.39					7.63	5.27		
C20:1 (11)			2.45	7.36	19.80	16.42	18.81	25.84	42.46	69.80	51.52
C20:2	0.39				11.66	10.12	12.46	16.18	24.59		27.89
∑FFA	99.92	707.8	748.03	1053	1958.2	1934.5	2478	3084	4186.97	5560.89	5569.9
∑SFA	32.41	301.9	497.5	521.2	670.3	791.6	984.5	1249	1386	1970.18	2104
∑MUFA	57.43	325.3	196.4	400	927.4	804.6	1092	1381	2212	2772.10	2712
∑PUFA	10.08	80.6	45.19	131.6	540.5	413.9	401.1	454.2	657.1	818.61	753.87
SFA / MUFA	0.564	0.928	2.533	1.303	0.723	0.984	0.901	0.905	0.626	0.711	0.776
SFA / PUFA	3.215	3.746	11.01	3.959	1.24	1.913	2.454	2.75	2.109	2.41	2.79

During the development of the fermentation which occurs at least during the first 6 days of the drying/smoking process (Sample 3), intrinsic and microbial lipolysis phenomena proceeded continuously up to day 180 (Sample 10), and was traduced on a 4 fold increase of the FFA level (1053 mg vs 4187 mg). Apart the initial phase where SFA content was still superior to MUFA (SFA/MUFA=1.3), the unsaturation level of the FFA profile gradually increased up reaching a SFA/MUFA relationship of 0.63 (55 days of ripening-S8). This is in agreement with the results reported by Chasco *et al*., (1993) for identical dry fermented sausages.

The conditions under which the processing stages take place are thought to be extremely important to the definition of the eating quality of the final product and to its stability over the storage period. Despite the formation of straight chain aldehydes butanal, pentanal and hexanal by a mechanism other than lipid peroxidation should not be ruled out, a significant correlation between their concentration, namely that of hexanal, and the rancid alteration of lipids has been observed (Shahidi *et al*., 1986).

From an extended study made on Paínho de Portalegre (results submitted for publication elsewhere) it could be observed that hexanal was never identified in all evaluated ripening periods (Table 3).

Table 3- Relative percentages % r.p. of volatile aldehydes detected along the drying/ripening stages.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
2-Metylpropanal	-	3.73	0.0	0.0	1.63	1.36	0.0	0.0	0.0	0.0
Butanal	-	0.0	0.0	0.0	0.0	0.0	0.39	0.0	0.69	0.48
2-Metylbutanal	-	0.0	0.0	0.0	0.0	0.20	0.15	0.19	0.62	1.65
Pentanal	-	3.45	0.0	0.3	0.39	0.0	0.15	0.0	0.82	0.0
2-Metylpentanal	-	0.0	0.94	0.0	0.0	0.27	0.17	0.0	0.0	0.0
Hexanal	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Pentanal and 2-metylpentanal predominated at the early phases of processing (spiced raw materials stored at a chilling room - 3.45%r.p.) and during fermentation (first 6 days on drying/smoking house -0.94%r.p.), but its concentration decreased during on going stages to less than 0.5%. The presence of butanal was only noted from day 40 of ripening in minor concentrations (0.39%, 0.69% and 0.48% at day 40, 120 and 180 of storage ripening period, respectively). The detection of 2-metylpropanal in S2, with a value of 3.7%r.p., is probably a consequence of the utilization of linoleic acid by microbial agents (Grosch, 1987) and could explain the drop observed for this fatty acid in the free form at this processing stage (80.6mg to 45.2mg).

Conclusions

Painho de Portalegre is characterized by a strong lipolysis during the processing and storage ripening period, over 180 days, traduced by an expressive liberation of fatty acids, which is more pronounced in the period of greater microbial development (first 15 days of drying/smoking stage). Excepting the earlier processing period in which saturated fatty acids predominated, MUFA is the most important class of compounds. The reduced production of straight chain aldehydes, namely hexanal, can be an indicator of good lipid stability, associated to the natural ingredients used on spicing and the intense/long smoking phase.

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