

Lipolytical changes in Dacia sausage, a Romanian dry cured sausage

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Abstract

Three batches of Dacia sausage were produced, one without starter culture (sausage A), one with starter culture consisting of *Lactobacillus sakei* and *Staphylococcus equorum* (sausage B), and one with starter culture consisting of *Lactobacillus sakei*, *Staphylococcus equorum* and *Lactobacillus acidophilus* (sausage C). Samples from each batch of sausages were taken at 0 days (mix before stuffing), and after 2, 4, 7, 14, 21 and 28 days of ripening. Lipolysis was observed during ripening by the increase in total free fatty acids levels, peroxide value and the acidity of the fat, in the non started sausage and the two started sausages. Lipolytic activities were detected in all batches, irrespective of the presence of the starter culture. At the end of ripening, no significant differences associated with the use of starter cultures were observed

Keywords: starter culture, lipolysis, dry sausages

1. Introduction

Fermented sausages are products that before consumption pass through a more or less prolonged process of drying-ripening. Dacia sausage is a Romanian fermented dry sausage, produced from raw pork, salt, pepper, garlic, species, additives and starter culture. The mix is stuffed into natural casings and then smoked, dried and ripened. During the ripening of fermented sausages various changes take place, leading to the final characteristics of the sausage. Lipids are abundant in fermented meat products and their changes influence the aroma and the flavor of the final product. The aim of this article is to study the changes in the lipid fraction during manufacture of Dacia sausage.

2. Materials and methods

Sampling. In order to carry out this study, three batches of Dacia sausage were produced in triplicate: one without starter culture addition (sausage A), one with starter culture consisting of

with *L.sakei* CECT 5964 and *S.equorum* SA25 (sausage B) and one with starter culture consisting of *L.sakei* CECT 5964, *S.equorum* SA25 and *L.acidophilus* CECT 903 (sausage C). From each batch of sausage, samples at 0 days (mix before stuffing), and after 2, 4, 7, 14, 21 and 28 days of ripening were taken. Each sample consisted of two entire units of Dacia sausage. After stuffing and a 24 hours drying period, the sausages were smoked in a smoking chamber. After smoking, the sausages were transferred to a drying-ripening chamber where they were kept for the rest of the ripening period.

Once collected, samples were transferred to the laboratory under refrigeration. For the analysis, the casings were removed and the edible parts were ground in a Moulinex mincer, until a homogenous mass was obtained. After microbiological analysis and determination of moisture content, water activity and pH, the samples were stored under freezing conditions, prior to further analysis.

Analytical methods. Extraction of fat was performed according to Folch, Lees, and Stanley (1957) [14]. The values of acidity of the fat were determined using the Spanish Official Standard UNE 50.011 [15]. Free fatty acids were separated from the triglycerides in polypropylene columns packed with NH₂-aminopropyl, following the procedure described by Antequera et al. (1994) [16]. The procedure described by Schlenk and Gellerman (1960) [17] with some modifications was followed for the methylation of the free fatty acids. The identification and quantification of the free fatty acids was performed by gas chromatography using a Trace GC (Thermo Finnigan, Austin, TX) chromatograph, equipped with a split/splitless AI 3000 Autoinjector and a flame ionisation detector. The separation of the different fatty acids was carried out using an Innowax column: 30 m long, 25 mm ID, 0.25 μ m film thickness (Agilent Technologies, Palo Alto, CA). The temperature of the detector was 250 °C and that of the injector 230 °C. The gases used were air (350 ml/min), hydrogen (335 ml/min) and helium (carrier gas) (30 ml/min).

A standard from Sigma Chemical Co. that contained the methyl esters of the following fatty acids was used: capric (C10); undecanoic (C11); lauric (C12); tridecanoic (C13); myristic (C14); myristoleic (C14:1); pentadecanoic (C15); cis-10-pentadecenoic (C15:1); palmitic (C16); palmitoleic (C16:1); margaric (C17); cis-10-heptadecenoic (C17:1); stearic (C18); oleic (C18:1 cis); elaidic (C18:1 trans); linoleic (C18:2); linolelaidic (C18:2 trans); linolenic (C18:3); arachidic (C20); cis-11-eicosenoic (C20:1); cis-11, 14 eicosadienoic (C20:2); cis-11, 14, 17-eicosatrienoic (C20:3); arachidonic (C20:4); heneicosanoic (C21); behenic (C22); erucic (C22:1); cis-13, 16 docosadienoic (C22:2); cis-4, 7, 10, 13, 16, 19-docosahexaenoic (C22:6); tricosanoic (C23); lignoceric (C24); and nervonic (C24:1).

This standard contained between 2 and 4% of each one of these fatty acids. All the samples and standards were injected at least in duplicate. Repeatability tests were performed by injecting a standard and a sample consecutively six times in a day. Reproducibility tests were also carried out by injecting the standard and the sample twice a day for three days under the same experimental conditions.

Significant differences ($p < 0.05$) were not found between the results obtained in these tests.

Statistical methods. In order to study significant differences between the different sampling points during the ripening process in the batches, a variance analysis (ANOVA) was performed, with a confidence interval of 95% ($p < 0.05$).

3. Results and Discussion

The changes in the fat acidity and peroxide during the manufacture of the three batches made with or without starter culture are shown in Figure 1. This value is the indicative of the degree of fat hydrolysis and of the free fatty acid content and they increased progressively in all the batches. The acidity of the fat increases from initial values of 1,81 mg KOH/g of fat in the non started batch, 2,89 in batch B and 2,59 in batch C to final values of approximately 9 mg KOH/g of fat. This represents a considerable increase which is in concordance with those described by other authors [6]. There were no significant differences in the acidity of the fat associated with the use of starter culture.

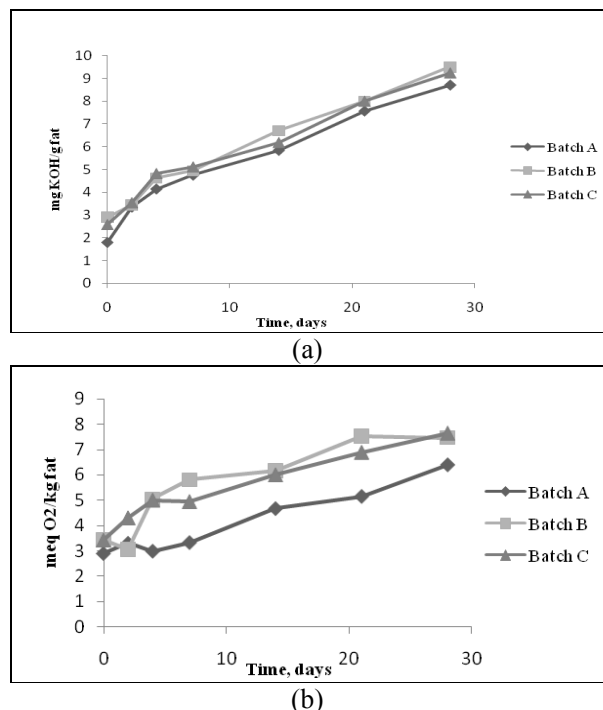


Figure 1. Changes in the fat acidity value (a) and peroxide value (b) in the three batches

The peroxide values were similar at the end of ripening process for the two batches with starter culture and smaller in the one without starter culture.

This parameter had a progressive increase reaching values of 6,38 in batch A, 7,45 in batch B and 7,65 in batch C at the end of ripening period. Our values were similar to those described by Leon et al. (1978) [8] in salchichon, higher than those reported by Fernandez-Fernandez et al. (1997) [5] in Galician Chorizo (4,84 meq O₂/kg of fat), but smaller than those observed by Franco et al. (2002) [6] in Androlla (18,9 meq O₂/kg of fat after 42 days of ripening). The fact that the peroxide value increased from the 7th day of fabrication, after the liberation of the fatty acids, confirms the fact that peroxides are produced in dry sausages from the fatty acids liberated by lipolysis in the early stages of ripening [2-4]. The evolution of the oxidative processes during sausage ripening was related to the availability of the oxygen and the production of hydrogen peroxides by lactic acid bacteria [11].

The changes in the content of the different free fatty acids during the manufacture of the batches processed without and with starter cultures are shown in Tables 1,2,3 and 4, respectively. The total average content of free fatty acids increased significantly ($p < 0.05$), from 191-265mg/100g of fat in the mix before stuffing to values of 1600 mg/100 g of fat in all batches at the end of the drying-ripening stage. The main free fatty acids in the three batches were : oleic (C18:1), followed by linoleic (C18:2), palmitic (C16); stearic (C18). All the batches showed an increase of free fatty acids during ripening in agreement with other authors [3,4]. During the manufacture of Dacia sausage, there was an increase in all of the free fatty acids, with oleic (C18:1) and linoleic (C18:2) being the main fatty acids released. The greatest increase in levels of these fatty acids took place during the drying-ripening stage.

Table 1. Evolution of the free fatty acids in the non started batch A

	A						
	0	2	4	7	14	21	28
C10	0,10±0,04	0,20±0,13	0,24±0,01	0,35±0,04	0,54±0,27	0,92±0,52	1,20±0,16
C12	0,26±0,07	0,29±0,05	0,27±0,04	0,39±0,11	0,66±0,31	1,01±0,48	1,20±0,36
C14	2,73±0,85	3,96±1,48	3,52±0,19	6,04±2,17	10,15±5,20	16,22±7,96	19,34±6,69
C14:1	0,07±0,05	0,07±0,06	0,05±0,02	0,07±0,06	0,12±0,03	0,19±0,03	0,23±0,01
C15	0,31±0,14	0,36±0,08	0,37±0,05	0,48±0,04	0,60±0,03	0,77±0,10	0,94±0,11
C15:1	0,09±0,09	0,14±0,14	0,09±0,05	0,19±0,14	0,17±0,12	0,33±0,27	0,38±0,04
C16	63,23±15,3	95,05±38,29	75,89±3,88	124,26±41,91	171,71±75,47	253,23±105,14	307,87±86,27
C16:1	5,12±1,52	6,43±1,60	7,30±0,48	11,98±4,41	18,55±7,74	28,75±9,06	35,49±10,46
C17	1,38±0,26	2,09±0,38	1,95±0,16	2,96±0,18	3,79±0,68	4,92±1,11	6,19±1,4
C17:1	0,64±0,32	0,81±0,17	0,86±0,24	1,34±0,32	2,02±0,95	3,46±0,99	4,07±0,94
C18	32,03±4,63	43,70±16,23	37,30±2,13	60,08±17,71	75,00±17,97	115,18±22,36	144,33±45,04
C18:1n9c	109,44±20,19	172,18±46,57	157,88±9,10	265,88±85,59	387,75±122,65	581,30±171,37	737,97±175,26
C18:2n6c	34,87±9,16	51,66±13,96	55,26±3,86	98,75±32,90	145,73±47,77	228,49±42,85	273,27±62,77
C18:3n6	0,28±0,09	0,39±0,16	0,44±0,04	0,71±0,14	0,98±0,31	1,39±0,36	1,68±0,47
C18:3n3	2,40±0,68	3,56±0,76	3,98±0,55	6,98±2,14	11,03±3,82	18,21±4,41	21,95±4,68
C20	0,32±0,06	0,32±0,06	0,33±0,02	0,59±0,16	0,65±0,44	1,18±0,52	1,34±0,34
C20:1n9	1,73±1,01	3,32±1,71	3,71±0,67	7,30±2,06	11,70±5,19	17,76±7,82	22,55±6,42
C20:2n6	1,46±0,18	2,37±0,35	2,40±0,18	4,74±1,98	7,56±0,75	10,18±2,24	12,58±2,44
C20:3n6	0,55±0,16	0,88±0,38	0,88±0,06	1,46±0,39	2,00±0,59	2,99±0,63	3,76±0,67
C20:3n3	0,33±0,07	0,51±0,16	0,53±0,06	1,07±0,32	1,84±0,57	3,02±0,52	3,60±0,70
C20:4n6	3,02±0,84	5,35±1,98	4,72±0,36	7,18±2,06	9,37±2,83	12,42±3,24	16,26±3,63
C20:5n3	0,03±0,03	0,09±0,05	0,06±0,05	0,11±0,00	0,21±0,05	0,33±0,06	0,35±0,06
C22	0,19±0,10	0,39±0,24	0,27±0,11	0,43±0,26	1,02±1,08	1,96±1,00	2,31±0,17
C22:1n9	0,65±0,64	0,44±0,26	0,71±0,78	0,50±0,59	0,76±0,56	0,71±0,59	1,83±0,20
C22:2	3,15±1,69	6,23±3,53	6,12±3,95	7,18±3,97	13,58±5,33	28,87±5,25	27,61±11,59
C23	0,35±0,13	0,91±0,05	1,04±0,11	1,74±0,93	1,54±0,54	3,48±1,19	3,171,39
C24	0,45±0,31	1,40±0,51	3,22±1,40	7,16±0,91	8,94±4,21	14,48±5,55	11,15±1,15
C24:1n9	0,04±0,02	0,12±0,04	0,09±0,01	0,18±0,13	0,19±0,06	0,21±0,05	0,37±0,21

Table 2. Evolution of free fatty acids in batch B produced with starter culture consisting of *Lactobacillus sakei* CECT 5964 and *Staphylococcus equorum* SA25

	B						
	0	2	4	7	14	21	28
C10	0,31±0,34	1,07±0,81	0,26±0,10	0,45±0,34	0,67±0,27	0,83±0,49	0,98±0,42
C12	0,25±0,08	0,26±0,06	0,33±0,06	0,52±0,20	0,67±0,31	0,86±0,42	1,04±0,32
C14	2,04±0,29	3,79±1,56	4,87±1,08	8,85±3,16	10,50±5,31	14,82±6,46	17,62±4,27
C14:1	0,30±0,42	0,24±0,26	0,18±0,05	0,16±0,10	0,18±0,18	0,16±0,06	0,21±0,04
C15	0,23±0,07	0,40±0,10	0,41±0,02	0,62±0,27	0,61±0,06	0,75±0,05	0,86±0,14
C15:1	0,36±0,44	0,12±0,06	0,15±0,11	0,28±0,16	0,19±0,16	0,24±0,15	0,38±0,09
C16	41,85±11,05	85,89±36,25	107,46±9,61	158,52±52,05	186,56±79,33	251,75±78,56	293,13±53,7
C16:1	4,15±0,49	5,88±1,73	9,00±2,21	16,37±6,09	21,34±8,51	29,12±8,75	34,68±8,31
C17	1,13±0,22	2,41±0,39	2,51±0,37	4,12±1,54	4,34±0,51	5,24±0,50	6,48±1,09
C17:1	0,48±0,25	0,76±0,07	0,82±0,16	1,93±1,69	2,15±0,49	3,16±0,65	3,55±1,09
C18	20,80±8,16	43,67±17,41	56,27±3,02	92,16±45,00	92,06±25,99	116,01±17,01	130,50±8,99
C18:1n9c	79,78±25,76	156,10±57,35	211,51±43,75	364,44±151,1	443,11±163,1	602,08±162,0	703,03±92,5
C18:2n6c	25,20±4,26	49,39±17,20	74,08±12,87	148,10±87,62	178,99±50,30	249,47±40,45	276,16±27,4
C18:3n6	0,18±0,06	0,41±0,11	0,56±0,05	1,08±0,67	1,25±0,28	1,50±0,23	1,76±0,28
C18:3n3	1,98±0,50	3,34±1,18	5,14±1,02	20,93±15,54	13,21±3,59	18,26±3,34	21,19±1,73
C20	0,23±0,06	1,16±0,76	0,57±0,05	0,89±0,31	1,16±0,37	1,23±0,40	1,41±0,28
C20:1n9	1,64±0,83	2,72±1,38	6,15±1,09	9,17±4,66	13,78±6,49	19,70±7,24	22,36±5,70
C20:2n6	1,67±0,81	3,00±1,34	3,65±0,38	7,61±4,10	8,41±2,84	11,77±2,38	13,42±1,21
C20:3n6	0,38±0,06	1,10±0,19	1,18±0,17	2,38±1,28	2,58±0,53	3,36±0,43	3,86±0,21
C20:3n3	0,26±0,04	0,57±0,25	0,88±0,20	2,06±1,23	2,46±0,62	3,25±0,57	3,86±0,13
C20:4n6	2,25±0,42	5,33±2,25	5,51±0,84	8,87±4,79	10,25±2,21	13,13±2,06	14,97±1,64
C20:5n3	0,08±0,11	0,16±0,06	0,09±0,08	0,21±0,08	0,26±0,07	0,32±0,05	0,40±0,04
C22	0,17±0,08	0,66±0,35	0,39±0,27	1,18±1,00	1,26±0,48	1,74±0,27	2,88±0,63
C22:1n9	0,431±0,33	1,34±0,51	0,66±0,28	0,76±0,31	0,72±0,25	0,46±0,11	0,50±0,29
C22:2	3,38±1,81	11,10±9,39	10,78±8,35	15,89±9,82	20,85±7,20	22,66±6,94	26,80±2,30
C23	0,50±0,19	1,18±0,68	1,62±0,56	2,15±0,76	3,78±1,80	3,49±1,06	3,85±0,49
C24	1,19±0,14	13,49±8,95	5,98±2,75	16,21±14,88	15,62±8,09	9,85±3,19	17,78±6,39
C24:1n9	0,67±0,12	0,98±0,66	1,16±0,96	0,22±0,09	0,30±0,08	0,41±0,12	0,39±0,11

Table 3. Evolution of free fatty acids from batch C with starter culture consisting of *Lactobacillus sakei* CECT 5964, *Staphylococcus equorum* SA 25 and *Lactobacillus acidophilus* CECT 903

	C						
	0	2	4	7	14	21	28
C10	0,16±0,10	0,22±0,02	0,35±0,36	0,79±0,56	0,36±0,24	0,73±0,33	0,92±0,35
C12	0,23±0,03	0,28±0,06	0,43±0,17	0,48±0,33	0,55±0,28	0,90±0,39	1,14±0,57
C14	2,20±0,71	2,75±0,07	6,09±3,48	4,30±0,19	5,91±0,90	10,60±1,97	13,92±1,23
C14:1	0,05±0,03	0,17±0,10	0,10±0,09	0,09±0,02	0,11±0,05	0,17±0,05	0,28±0,06
C15	0,21±0,05	0,34±0,04	0,37±0,18	0,48±0,10	0,48±0,06	0,72±0,11	1,01±0,15
C15:1	0,15±0,11	0,56±0,91	0,10±0,08	0,12±0,14	0,04±0,02	0,09±0,05	0,08±0,05
C16	62,15±2,19	64,57±2,77	104,72±32,49	90,88±4,04	119,66±12,81	190,92±47,05	253,16±12,47
C16:1	4,32±0,81	6,86±2,38	9,83±3,74	8,27±1,49	12,28±1,36	22,23±5,80	30,56±7,35
C17	1,19±0,22	1,71±0,09	2,35±0,03	2,84±0,39	3,52±0,40	5,13±1,17	7,84±1,24
C17:1	0,42±0,07	0,91±0,03	1,37±0,92	1,22±0,53	1,70±0,98	3,20±0,91	4,74±1,78
C18	28,63±5,15	40,52±10,05	67,38±3,61	66,16±22,31	93,76±32,95	127,30±13,33	131,51±3,67
C18:1n9c	109,45±14,25	114,06±4,61	220,68±93,98	278,58±149,91	480,99±244,73	661,53±194,58	653,77±80,11
C18:2n6c	32,77±5,79	31,91±7,14	76,86±19,44	69,23±8,56	112,16±15,51	199,32±43,26	290,63±57,78
C18:3n6	0,20±0,05	0,28±0,08	0,48±0,14	0,68±0,09	0,92±0,20	1,38±0,27	2,25±0,60

C18:3n3	2,23±0,40	1,97±0,47	5,40±1,42	4,85±0,50	13,36±1,70	14,84±2,85	21,78±3,33
C20	0,28±0,07	0,29±0,02	0,64±0,22	0,69±0,35	0,83±0,46	1,22±0,46	1,20±0,04
C20:1n9	2,09±0,67	2,86±0,07	9,32±1,21	7,98±2,34	7,26±2,31	13,63±6,29	17,72±3,35
C20:2n6	1,66±0,02	1,85±0,07	3,66±0,78	3,03±0,50	4,83±1,10	12,84±2,36	13,06±2,36
C20:3n6	0,42±0,10	0,71±0,08	1,09±0,18	1,43±0,38	1,54±0,36	3,16±0,02	5,08±0,48
C20:3n3	0,42±0,08	0,84±0,16	1,09±0,73	1,43±0,67	1,54±0,95	3,48±0,91	4,39±1,56
C20:4n6	2,47±0,40	4,43±0,19	5,50±0,16	6,18±0,52	9,71±0,67	12,45±2,18	18,16±1,17
C20:5n3	0,04±0,02	0,08±0,02	0,11±0,00	0,12±0,02	0,20±0,04	0,38±0,07	0,54±0,03
C22	0,16±0,01	0,17±0,02	0,58±0,26	0,64±0,06	0,71±0,35	1,74±0,62	2,19±0,24
C22:1n9	0,47±0,00	0,46±0,20	0,43±0,13	0,36±0,04	0,52±0,26	0,74±0,01	0,70±0,25
C22:2	4,48±3,67	8,96±7,95	10,49±6,39	12,99±9,01	22,32±8,47	17,19±3,66	27,07±0,00
C23	0,67±0,06	1,28±0,41	1,56±0,30	1,54±0,97	1,59±0,51	2,36±1,27	6,76±1,20
C24	1,26±0,37	4,60±0,96	12,45±3,31	11,51±1,23	13,42±4,38	13,21±3,00	29,32±2,60
C24:1n9	0,05±0,01	0,16±0,02	0,45±0,44	0,19±0,09	0,19±0,09	0,34±0,11	0,40±0,08

Table 4. Distribution of free fatty acids in the three formulations

Batch	Days	FFA (mg)	MONO	UNSATURATED	SATURATED	POLY
A	0	265,36±53,02	117,81±20,54	163,95±32,28	101,40±21,27	46,13±12,26
	2	403,38±120,17	183,55±49,47	254,64±63,89	148,74±56,34	71,09±14,42
	4	369,60±16,64	170,73±8,47	245,15±14,00	124,45±4,62	74,42±8,22
	7	620,22±190,88	287,47±92,99	415,69±128,80	204,53±62,78	128,21±36,35
	14	888,29±289,86	421,30±136,94	613,63±195,05	274,65±101,67	192,33±61,20
	21	1352,12±375,58	632,76±189,16	938,70±237,80	413,41±144,31	305,94±57,15
	28	1663,13±402,31	802,93±193,00	1164,04±272,33	499,08±140,09	361,11±85,58
B	0	191,81±49,71	87,64±27,72	123,06±30,37	68,75±19,78	35,41±5,30
	2	396,44±157,66	167,96±60,77	242,40±92,05	154,03±65,91	74,43±31,64
	4	512,87±78,29	230,27±45,77	332,17±65,19	180,69±13,30	101,90±23,25
	7	887,69±367,49	394,80±161,93	601,98±267,94	285,71±100,91	258,36±82,22
	14	1037,36±359,84	481,80±178,22	720,09±242,53	317,27±118,37	273,39±41,29
	21	1385,75±327,11	655,36±178,21	979,12±224,75	406,63±103,88	323,75±55,03
	28	1604,17±195,59	765,13±106,82	1127,58±135,32	476,58±62,97	362,45±29,27
C	0	218,39±77,06	97,36±35,94	137,24±47,49	81,15±31,13	39,87±11,59
	2	339,56±105,93	151,37±44,98	209,41±66,79	130,14±39,14	58,03±22,10
	4	528,23±125,49	182,33±121,35	320,28±100,24	207,94±57,31	137,95±54,53
	7	548,17±321,40	213,37±191,85	343,14±248,53	205,03±76,47	129,76±62,69
	14	897,13±474,06	417,44±260,27	625,24±352,65	271,88±121,42	207,80±92,40
	21	1360,42±480,82	624,62±225,71	953,49±351,03	406,92±131,29	328,86±125,34
	28	2060,50±897,47	978,47±472,87	1459,63±630,36	600,87±268,44	481,16±158,02

There were no significant ($p < 0.05$) differences between the three batches, suggesting that use of starter cultures is not affecting the fatty acids profile.

The final value for each individual fatty acid is the result of the balance between its release from glycerides and phospholipids and its oxidative degradation.

Maturation brought a widespread increase in the free fatty acids content, all main fatty acids increasing to a significant extent but the phenomenon was more marked for the unsaturated ones.

Lipases attack preferentially the fatty acids placed in the outer position of the triglycerides molecules, and position 3 more than position 1, where the unsaturated acids are predominantly placed.

The lipolytic activity was ascribed to meat tissue lipases than to lipolytic starters, such as micrococci, the last being credited for the reduction of nitrates and nitrites [7-13].

The role played by additives and technological conditions in the liberation of fatty acids was studied by Moltiva and Toldra (1993) [10] and they found that the acid lipase activity is activated by decreasing water activity and by increasing salt concentration. Stahnke (1995) didn't share the latter point who reported a significant negative effect of salt on fatty acids values [12].

Differences in lipolytic activity could derive also from the presence of surface moulds [1].

The analysis of the results obtained with the three formulations produced in this study, and of the data available in the literature, confirms that lipolysis in dry fermented sausages is mainly due to endogenous lipases.

The differences between formulations could be due to inherent variations of the lean and fat tissues used.

Endogenous lipases activity is favoured by low pH, but free fatty acids production is stimulated by high temperatures.

The minces of the three formulations were characterized by higher contents of unsaturated fatty acids, no significant differences being observed in the monounsaturated content. In saturated content we can observe differences between the three sausages, contents in sausage B being significantly smaller than in sausages A and C.

The free fatty acid content and profile were not significantly affected by the use of starter cultures. The three formulations, as it can be observed from the distribution of the main groups (saturated, monounsaturated and polyunsaturated), do not differ appreciably.

4. Conclusion

The different formulations used to produce the three batches have provided data which support the hypothesis that most of the lipolysis taking place during maturation is of endogenous origin. Other technological parameters such as: additives, spices, starter cultures have no perceivable effects on lipolysis and lipid oxidation.

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