UNIVERSITY OF
"DUNĂREA DE JOS"
GALAŢI

FACULTY OF FOOD SCIENCE AND ENGINEERING

UTILIZATION OF STARTER CULTURES IN FERMENTED MEAT PRODUCTS

(ABSTRACT)

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Către

Universitatea " Dunărea de Jos " din Galați vă face cunoscut că în data de *07.11.2011*, ora *10.00*, în *sala Senatului Universității "Dunărea de Jos" din Galați*, va avea loc susținerea publică a tezei de doctorat intitulată: *"UTILIZAREA CULTURILOR STARTER ÎN PRODUSE DIN CARNE FERMENTATE"*, elaborată de domnul/doamna *ing. SIMION ANA-MARIA(CIUCIU)*, în vederea conferirii titlului științific de doctor în Domeniul de doctorat - *Inginerie industrială*.

Comisia de doctorat are următoarea componență :

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Cu această ocazie vă transmitem rezumatul tezei de doctorat și vă invităm să participați la susținerea publică. În cazul în care doriți să faceți eventuale sprecieri sau observații asupra conținutului lucrării, vă rugăm să le transmiteți în scris pe adresa Universității, str. Domnească nr. 47, 800008 - Galați, Fax - 0236 / 461353.

RECTOR,
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SECRETAR DOCTORAT,
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Contents

Foreword

"It is unbelieveable that such a tiny streamlet, as was our initial test trial with the first bacterial starters exactly 40 years ago, grew to a big river for an important industry. Who would have believed then that the use of starter cultures would be a matter of course today." (F.P. Niinivaara 1994)

Meat and meat products are the result of the need to preserve meat, and the science of producing dry fermented sausages is known from ancient times.

Preservation by reducing water activity, combined with the pH drop may be considered the most ancient technology. Products were dried with natural circulation of air and reduction of water activity by salting was made by imersion or by whipping the meat surface with big salt cristals. If fermentation was involved, it was due to autochtonous flora.

Before '40 fermentation was realised by inoculation of a fermented meat quantity in the fresh mix. The result wasn't always the expected one, the final product beeing frequently inadequate from acidity point of view. In low acidity products, spoilage bacteria were able to develop, having negative consequences on products quality and its safety.

When the need for bigger quantities of meat products grew up, after the World War II, researchers began developing starter cultures for meat products with the aim to ensure a standard quality for the fermentation process. The idea to use lactic acid bacteria cultures, in dry cured sausages, appeared in USA, in '40 and the aim was to reduce maturation period and to ensure the sausage quality and flavour. The first lactic acid bacteria culture, introduced as a pure *Pediococcus cerevisiae* culture, appeared in 1955 in USA and in the same time *Micrococcus M53* appeared. Later, in 1964, micrococaceae were combined with *Lactobacillus plantarum.*

In our days, many companies are producing pure cultures of Lactobacillus spp., *Pediococcus acidilactici, Pediococcus pentosaceus, Staphylococcus xylosus, Staphylococcus carnosus* or these strains mixes for meat products fermentation.

Use of starter cultures aim is to ensure the standardization of the production of dry sausages, obtaining high sensory and organoleptic quality products and improvement of the final product quality.

This practice comes to meet of the principles of the eficiency and food safety, by reducing the maturation step which leads to a smaller imobilization of spaces, current assets and a reduced consumption of utilities.

The PhD thesis *Utilization of starter cultures in fermented meat products* main objective is to obtain and to study the effects of two starter cultures containing *Lactobacillus sakei, Staphylococcus equorum* and *Lactobacillus acidophilus* on the physicochemical, biochemical and sensory parameters involved in the characterisation of three batches of Dacia sausage, produced with and without starter cultures.

In this context, the doctoral program studies focused on the following objectives :

- Selection of the microorganisms which were used as starter cultures to produce Dacia dry sausage;
- Production at a pilot scale of the three batches, two with starter addition and one as control;
- Analyze the evolution of most important microbial groups present in the produced Dacia batches ;
- Study of the evolution of physicochemical and biochemical parameters involved in the production of the three batches;
- Total fatty acids and free fatty acids profile determination with gas chromatography;
- Biogenic amine content determination in the three batches by HPLC ;
- Volatile compounds profile determination of the three batches by gas chromatography coupled with mass spectrometry;
- Sensory analysis of the three Dacia sausages.

To carry out the research, according to the scientific objectives of the PhD thesis, a modern research infrastructure was used:

- Laboratory of Food Technology, Faculty of Sciences (University of Vigo), Ourense, Spain

PhD THESIS STRUCTURE

The PhD manuscript is presented on 197 pages and its divided in three parts as follows:

I. Literature review, **II. Materials and methods** and **III. Results and discussions**. The manuscript contains 44 figures and 10 tables.

The first part is divided in seven subchapters and describes the technology of dry fermented sausages, the effect of adding starter cultures to the sausages, the parameters that influence the activity of starter cultures and the physicochemical and biochemical changes that take place in the sausages during drying-ripening period.

The second part is divided in three subchapters and describes the methodology, reagents and materials used in this thesis. Here we can find the description of the extraction and identification techniques for the fatty acids, volatile compounds and biogenic amines.

The third part is divided in five subchapters that present the results obtained in this PhD thesis and the partial conclusions. The last subchapter presents the general conclusions of the thesis.

Chapter 2
MATERIALS AND METHODS

2.3.1. Fabrication recipe and technological parameters for Dacia dry sausages production

Fabrication recipe used to produce the three batches comprises:

- Lean pork 80 %;
- Fat 20 %;
- Salt 2,65 %;
- $NaNO_3$ 0,075 %;
- Garlic 0,035 %;
- Sugar 0,180 %;
- White pepper 0,260 %;
- Allspices 0,075 %.

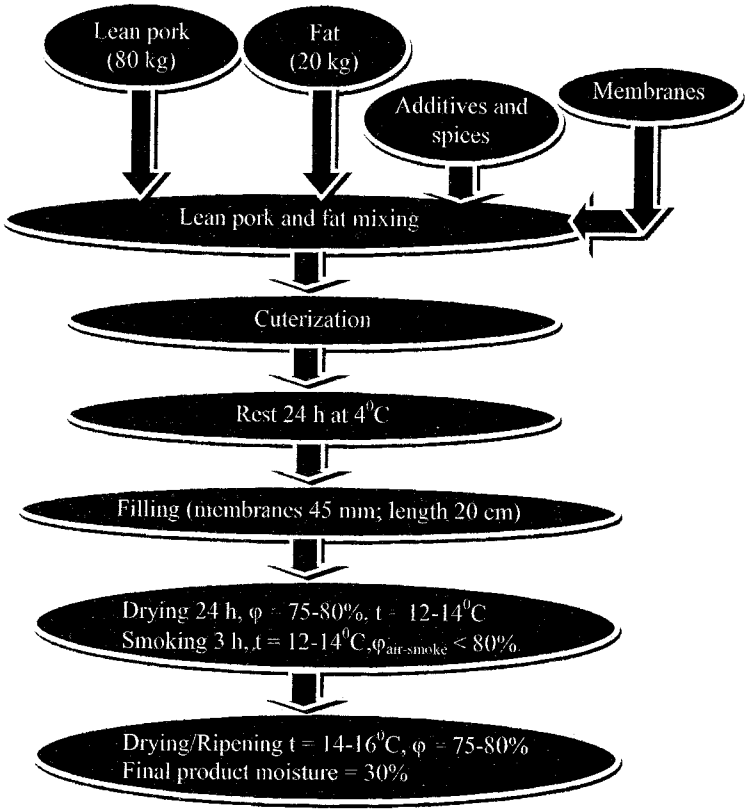

Figure 1 ***Technological parameters and steps in Dacia sausage production***

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.

Chapter 3
RESULTS AND DISCUSIONS

3.1. Bacterial strains selection

Based on the literature review, we chosed a *Lactobacillus sakei* strain, which is frequently used in fermented sausages. As an original element we decided to combine this strain with a strain of *Lactobacillus acidophilus* and a *Staphylococcus equorum* strain in one starter and only with *Staphylococcus equorum* in the second starter culture.

The *Lactobacillus* strains were provided by The Spanish Strains Collection and were : *Lactobacillus sakei 5764* and *Lactobacillus acidophilus 903*. The *Staphylococcus equorum* strain was isolated in the Food Technology Laboratory, Faculty of Sciences, Ourense, Spain from Androlla, traditional fermented sausage from Galicia.

In order to establish the dimension of the inoculum growth curves and acidification curves were performed for the *Lactobacillus* strains, also growth curves were performed for the *Staphylococcus* strain.

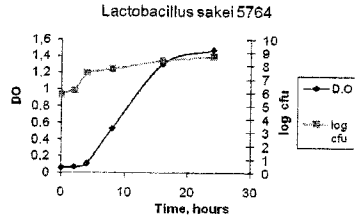

Figure 3.1. ***Growth curve for Lactobacillus sakei 5764***

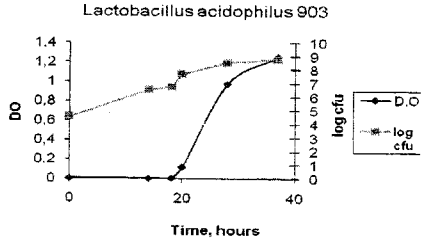

Figure 3.2. ***Growth curve for Lactobacillus acidophilus 903***

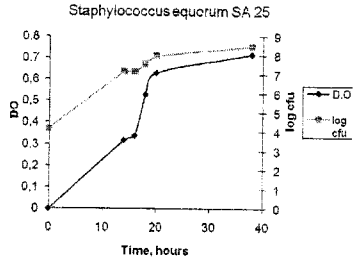

Figure 3.3. ***Growth curve for Staphylococcus equorum SA 25***

We produced three batches of Dacia sausage, in triplicate, as it follows: one without starter culture (control) – batch A, one with starter culture consisting of *Lactobacillus sakei* and *Staphylococcus equorum* – batch B and one with starter culture consisting of *Lactobacillus sakei*, *Staphylococcus equorum* and *Lactobacillus acidophilus*.

The added quantity was acording the literature review, in order to achieve a bacterial population of 10^7 c.f.u/g for *Lactobacillus sakei* and *Staphylococcus equorum* and 10^8 c.f.u/g for *Lactobacillus acidophilus*.

3.2. Evolution of the microflora

The evolution of the dry fermented sausages flora was evaluated during the whole production process by enumeration of the total aerobic mesophilic flora, lactic acid flora, micrococaceae and enterobacteria.

3.2.1. Total aerobic mesophilic flora

The evolution of this microbial group is shown in figure 3.5, for the three batches. As we can see the evolution of total viable mesophilic counts was similar for the three batches, although some differences have been observed. The bacterial population was higher for the inoculated batches (B and C) than in the control sausage during the whole process.

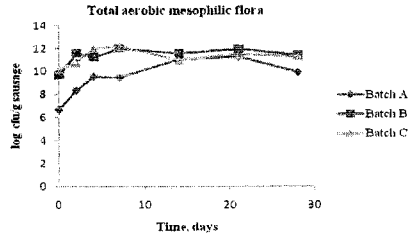

Figure 3.5. ***Evolution of the total aerobic mesophilic flora counts during fabrication process***

In control batch (A), the viable counts of aerobic mesophilic flora showed smaller values during the entire fabrication process, than in the inoculated batches. Our results were one

logarithmic unit higher than those reported by Metaxoupoulos et al. (2001) and similar to those reported by Gil and Newton (1977) for the initial counts of dry sausages, with a medium value of 6 log c.f.u/g.

The total aerobic bacteria counts in the final product were about 11.3 log c.f.u/g for the inoculated batches and 9.89 log ufc for the control batch.

3.2.2. Lactic acid bacteria

Lactic acid bacteria (LAB) are very important because they produce sugar fermentation which results in organic acids production, causing the pH drop, an important process for the sensorial quality, characteristic texture and microbial safety of the final products (Castaño et al., 2002; Cenci-Goga et al., 2008).

The evolution of lactic flora for the three batches during the whole fabrication process is shown in figure 3.6 .

As we can see the lactic bacteria counts were significantly higher in the inoculated batches, being around 10 log c.f.u/g of sausage. These values were about four units higher than in control sausage (6 log c.f.u/g of sausage).

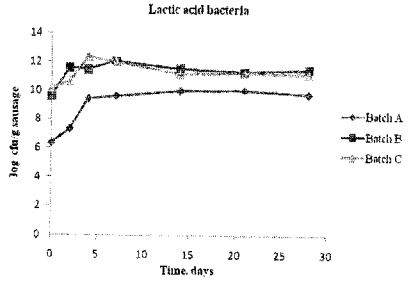

Figure 3.6. ***Evolution of lactic acid bacteria counts during fabrication process***

Various researches assumed that starter cultures inoculation increases the initial lactic bacteria counts (Garciá et al., 1992; Huang and Lin, 1995; Sanz et al., 1997a). High initial counts indicate that the inoculation was correct.

As expected, counts of lactic bacteria increased during the first 7 days, which coincides with fermentation phase, then a stabilization was observed and a small decrease tendency in the last days of fabrication.

Our results, in the non inoculated batch, were similar to those reported by Samelis et al. (1994), in naturally fermented sausages. The dominance of lactic acid bacteria, in sausages produced without starter culture, was previously described by other authors (Selgas, et al., 1988; González and Diez, 2002; Garciá-Fontán et al., 2007; Ferreira et al., 2009; Elias and Carrascosa, 2010).

Evolution of LAB, during Dacia sausage fabrication, coincides with those reported by other authors during different dry sausages manufacture (Metaxopoulos et al., 2001; González and Diez, 2002).

In some sampling points, LAB counts were superior to the total aerobic bacteria counts. This fact, impossible in practice, considered as an indicator of the absolute dominance of lactic acid bacteria, was also observed by other authors (Metaxopoulos et al., 2001; González and Diez, 2002; Garciá-Fontán et al., 2007), which utilized MRS agar as a culture medium.

Our results for LAB counts, is three logarithmic units higher than those obtained in other previous works (Latorre-Moratalla et al., 2007; Marcos et al., 2007; Ferreira et al., 2009; Baka et al., 2011), this fact being explained by the initial counts in the control sausage.

3.2.3. Micrococaceae

In this study, Micrococcaceae counts were about 5 log c.f.u/g in the inoculated batches and 4 log c.f.u/g in the control sausage. The reduction of this microbial group during the fermentation is probably due to the application of smoking on the 4th day and rapid pH reduction. This group of microorganisms is sensitive to smoke components, but acidification is considered the most important, inhibitory factor in dry sausages (Samelis et al., 1998).

These counts were inferior to those of the total aerobic flora, reflecting the competitiveness of these microorganisms in presence of acid-resistant bacteria in phase of active growth (Lücke, 1984).

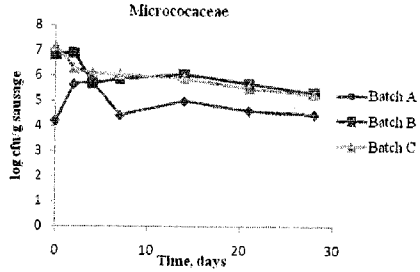

Figure 3.7. ***Evolution of micrococaceae counts during fabrication process***

The final results, for non inoculated batch are similar to those obtained by Benito et al. (2007) and Martin et al. (2007) in spanish dry sausages. Results for inoculated batches were similar to those obtained by González and Diez (2002).

3.2.4. Enterobacteriaceae

Initial Enterobacteriaceae numbers (10^2-10^3CFU/g) were similar in all batches and in the range usually reported for dry cured sausages (Sanz et al., 1997a) . Evolution of this microbial group is shown in figure 3.8.

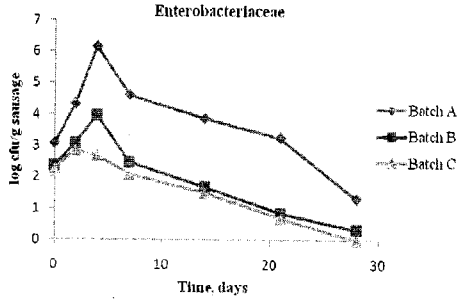

Figure 3.8. ***Evolution of enterobacteriaceae counts during fabrication process***

The initial low counts, for this group in all batches showed the hygienic quality of the meat, a good manufacture process control and a corresponding hygiene for the equipments and for those involved in the fabrication. The evolution of these microorganisms during ripening was different between formulations.

The batches inoculated had a lower number of *Enterobacteriaceae* after the 14th day of ripening, disappearing at the final of the ripening period.

The decrease in pH may partly explain the reduction and disappearance of these groups of bacteria. However, not only the pH is responsible for this inhibition because at the same pH the Enterobacteriaceae did not disappear until day 28 in non-inoculated batch.

The antimicrobial effect observed in inoculated sausage appears to be due to other compounds besides the low level of pH. We can add the moisture content, water activity and NaCl values.

Results obtained in this study are similar to those obtained in other studies (Garciá-Fontán et al., 2007; Casaburi et al., 2008; Baka et al., 2011).

3.2.5. Conclusions

Microbial strains combinations proved to be efficient in Dacia dry sausage production. Their use represents a guaranty for the safety and standardization of fermented sausages from microbiological point of view.

Lactic acid bacteria represented the dominant microflora for the sausages during the entire production period. Their survival and multiplication represent a guarantee for the proper acidification of the sausage formulation, inhibition of the undesirable flora and for the sensory characteristics development of fermented sausages.

In the inoculated sausages were created inadequate conditions for enterobacteriaceae survival, which population decreased under detection limit at the end of ripening time.

Chapter 4
PHYSICOCHEMICAL PARAMETERS

4.1. Moisture

We can see a progressive decrease of moisture, from initial values of approximately 61 – 63% till final values of 30 - 31%. The medium initial values, are similar to those reported by other authors in dry sausages formulations (Barranco Sánchez et al., 1985; Franco et al., 2002) .

The decrease in moisture content began immediately after membrane filling, the speed of this process being higher in the first 4 days, mostly for the inoculated batches. So the daily losses in moisture, in this period, were 2,42% for batch A, 2,98% for batch B and 3,16% for batch C. After this period, the drying process was relatively uniform.

Figure 4.1. shows the evolution of this parameter in the three batches during the fabrication period.

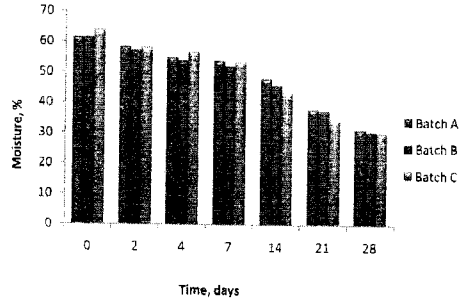

Figure 4.1. ***Evolution of moisture content during fabrication process***

The influence of starter cultures on this parameter wasn't statistically significant ($p > 0,05$).This fact may be explained by the same drying and ripening parameters for the three batches, the same salt content and the same caliber. This evolution of moisture has been

observed by Latorre-Moratalla et al. (2007), no differences being observed between inoculated batches and the non inoculated ones.

4.2. pH

Figure 4.2 shows the pH evolution in the three batches of Dacia sausage, during the fabrication period.

During the entire fabrication process the pH values for the inoculated batches were almost equal. This situation may be explained by the fact that *Lactobacillus* strains adapted very well to meat matrix.

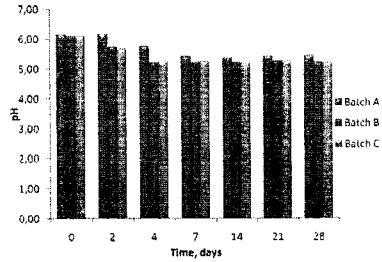

*Figure 4.2. **pH evolution during fabrication process***

The initial values were about 6.09 – 6.14 and our values were superior to those reported by other authors (González-Fernández et al., 2003), but similar to those reported by Franco et al. (2002). Starting with the 14th day of drying-ripening, when the maximum pH drop was reported, the pH values presented a low increase.

The LAB growth in the first stages of fermentation had a benefic effect on the pH drop of the started batches, which lead to disappearance of the undesirable flora.

The final pH values were situated between 5.19 and 5.20, for the started batches, and 5.42 for the non started batch, pointing out the beneficial effect of starter cultures on acidification. These results are similar to those reported by Olesen et al. (2004) and Cenci-Goga et al. (2008).

4.3. Water activity

Water activity values decreased from initial values of 0.96 to final values of 0.82 - 0.83. We didn't find any statistical significant differences ($p > 0.05$) between the three batches. Figure 4.3. shows the evolution of this parameter for the three batches.

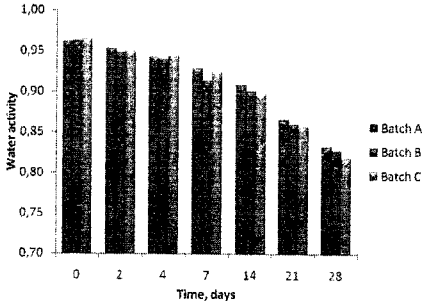

*Figure 4.3. **Evolution of water activity during fabrication process***

The evolution of this parameter coupled with the pH drop lead to disappearance of the spoilage bacteria. This parameter represents one of the factors participating at the microbiological stability of dry sausages, having a greater influence as maturation process advances (Leistner, 1987).

4.4. NaCl content

The salt content, expressed as NaCl/100 g of sausage, increased during the drying – ripening period, this evolution resulting from the moisture lose during processing. At the end of ripening (day 28) the sodium chloride reached values of approximately 4 g/100 g of sausage.

Figure 4.4. shows the evolution of this parameter during the fabrication process.

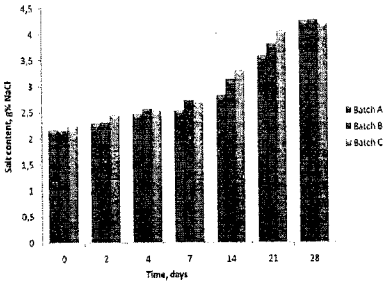

*Figure 4.4. **Evolution of salt content during fabrication process***

If we report the salt content at 100 g of dry matter, we can see that the salt content was constant during the fabrication of Dacia sausages, no significant differences ($p > 0.05$) between batches being observed.

4.5. Total protein content

Figure 4.5. shows the evolution of protein content in Dacia batches during the entire fabrication process. Values are expressed as g of protein/100 g of sausage.

As we can see the protein content increased significantly till the end of ripening, this evolution being favored by the dehydration process which lead to a dry matter concentration.

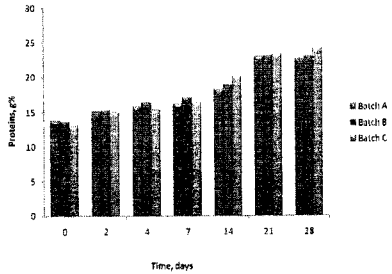

*Figure 4.5. **Evolution of protein content during fabrication process***

If we consider the protein as g/100 of dry matter, we see that the content remained constant during the entire process. There were no significant differences ($p > 0,05$) among batches and between different stages of the drying – ripening process. Final values were about 32 – 34 g of protein/100 g of dry matter.

Our results are significantly higher than those reported by Salgado et al. (2005), for the industrial chorizo and those reported by Zanardi et al. (2004) for different sausages formulations and different starter cultures.

4.6. Lipid content

The evolution of lipid content in Dacia batches, expressed as g of fat/100 g of sausage is shown in figure 4.6.

Fat levels increased during the fabrication process, from initial values situated between 19.35 and 20.87 %, till final values of 37.03 – 37.98 %.

There are some differences among batches but this could be due to sex, dietary fat composition, slaughtered weight, anatomical location, backfat thickness.

If we talk about fat levels, expressed as g of fat/100 g of dry matter, we can see that the lipid content was similar in the three batches during the entire process.

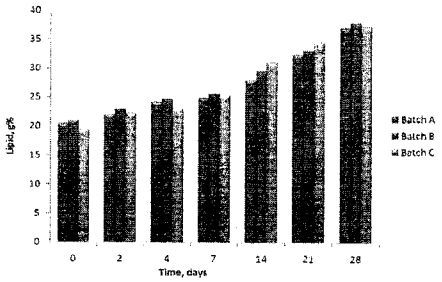

Figure 4.6. ***Evolution of the lipid content during fabrication process***

Our results are inferior to those obtained by Salgado et al. (2005) and superior to those reported by Zanardi et al. (2004). These differences may result from the variations in moisture content, a smaller fat value can be observed at a higher moisture content.

4.7. Titratable acidity

Lactic acid accumulation in the first fermentation days and later in the drying – ripening steps is related with the multiplication of lactic acid bacteria from the starter culture and those which are not inhibited by salt presence. In figure 4.7. the evolution of this parameter during the production process of the three batches of Dacia sausage.

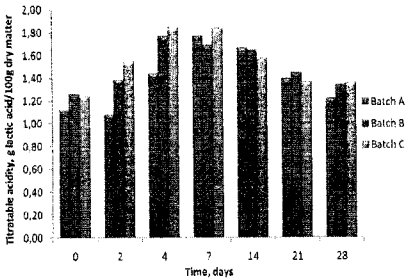

Figure 4.7. ***Evolution of titratable acidity during fabrication process***

This parameter increased in the first 7 days and then a decrease was registered till the end of process. As the dry matter content increased, the lactic acid production decreased and one of the factors influencing the lactic acid content was the content in free water of the product.

The evolution of this parameter was also influenced by drying – ripening temperature and the added sugar. Only few informations are available in the literature concerning this parameter and the processes involved in its evolution.

The decrease after the 14^{th} day could be related with the evolution of the flora able to consume lactic acid.

4.8. Nitrates content

In figure 4.8. we can see the evolution of this parameter during the entire fabrication process for the three Dacia batches.

As we can see the nitrate degradation is a continuous process, which began at the addition moment, due to the enzymatic equipment of meat and the one produced by the added starter culture.

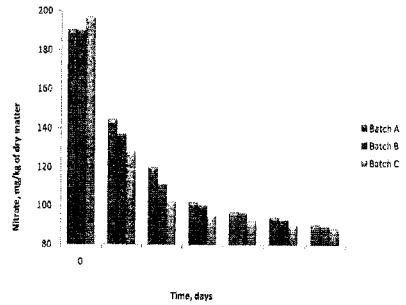

Figure 4.8. ***Nitrate content evolution during fabrication process***

This degradation process is influenced by the high pH of the sausage mixes and the presence of positive nitrate - reductase producing bacteria. A reduction of the degradation rate after 7 days of ripening, coincides with the pH decrease, which lead to the inhibition of nitrate – reductase because the substantial multiplication of lactic acid bacteria in batches B and C. in batch A, nitrate degradation continued with a relative high rate, because of the lower decrease rate of the pH.

Our results coincide with those reported by Navarro et al. (2001), levels of nitrate at the end of the process being low. The lowest level of nitrate were determined in batch C (88.3 mg/kg of dry matter) and B (88.9 mg/kg of dry matter), this fact may be attributed to the presence of *Staphylococcus equorum*. No significant ($p > 0.05$) differences were demined between inoculated batches.

4.9. Biogenic amine content

Amines were determined by high performance liquid chromatography (HPLC) method described by Eerola et al. (1993). Separation was performed using a reverse phase column C_{18} Teknokroma mod. Kromasil 100 (4,6 mm diameter and 25 cm length). The separation phase is based on previous extraction of amines, with 0,6 N perchloric acid, from 5 g of sample. The method allows to quantify, using an internal standard (1,7-diaminoheptane) procedure, tyramine, histamine, tryptamine, phenylethylamine, putresceine, cadaverine, spermidine and spermine.

The biogenic amine content in Dacia batches during the production process is shown in table 4.2. Data variations may be explained by the existence of several factors which can influence biogenic amine production, such as: hygienic quality of raw materials, type of starter culture, technological conditions and microbial charge brought in the elaboration and storage processes.

From a quantitative point of view the most important amine, found in the formulations before filling, was tyramine. It had significantly higher values ($p < 0,05$) in batch A (without starter addition). In final products, tyramine is commonly the most important amine (Talon et al., 2008) found in fermented sausages. In this batch, tyramine, could be related to tyrosine – carboxylase activity of wild lactic fermenting flora.

Tyramine is the most toxic amine, toxicological level being 100 – 800 mg/kg (Silla-Santos, 1996).

The addition of the starter cultures decreased the tyramine levels in the inoculated batches with approximately 50%, probably because the inhibition of enterococci, the main bacterial group associated with tyramine formation together with lactobacilli (Bover–Cid et al., 2001; Suzzi and Gardini, 2003).

This amine was followed by cadaverine in batches A and C, while in batch B it was followed by putresceine. Cadaverine and putresceine increased during production process, their formation being related with lysine and ornithine decarboxylation by enterococci and enterobacteria (Halász et al., 1994; Bover-Cid et al., 2001b). Martin et al. (2006) reported that both di-amines, putresceine and cadaverine, were simultaneously produced by micrococci. In

this way micrococci may represent one of the factors leading to an increase in cadaverine and putresceine levels during Dacia sausages fabrications.

Histamine is always associated with the presence of di-amines, especially cadaverine, which is consistent with the fact that these two amines are produced mainly by enterobacteria (Bover-Cid et al., 2000; Latorre-Moratalla et al., 2006). This amine had an increasing evolution during the fabrication process, reaching final values of 20.41 mg/kg in batch A, 18.58 mg/kg in batch B and 15.07 mg/kg in batch C.

Histamine is the only amine subjected to legal regulations, in some some fish species, with an upper limit of 100 mg/kg in Europe (EC, 2005). There are no limitations for fermented sausages, some authors suggesting 100 mg of histamine/kg of product as a limit for a potential risk for healthy persons (Brink et al., 1990).

Spermidine levels significantly increased ($p < 0,05$), while spermine remained constant throughout the manufacturing process for the three batches. These two physiological polyamines amines are natural meat microcomponents (Bardócz, 1995; Hernández-Jover et al., 1997a). Although they are always present in meat products (Cantoni, 1995; Bover-Cid et al., 1999), there is little information about their evolution during dry sausages production.

Suzzi and Gardini (2003) reported tryptamine values under 50 mg/kg, which coincides with values determined in this work (38 % in non inoculated batch, 22,66 % in batch B şi 9,79 % in batch C).

Low levels of phenylethylamine were also found in this study, results being similar to those reported by Paulsen and Bauer (1997) and Ruiz-Capillas et al. (2007), very low levels of this amine and rarely above 50 mg/kg.

Phenylethylamine is always associated with high levels of tyramine, this relationship being attributed to the fact that microorganisms with strong tyrosine-decarboxylase activity also have moderate capacity to decarboxylate phenylalanine (Bover-Cid, Izquierdo-Pulido and Vidal-Carou, 2001).

Spices might have been vehicles for potentially aminogenic microorganisms to meat batter or eventually amino acid-decarboxylase enzymes, especially pepper (Bover-Cid et al., 1999) and garlic powder (Latorre-Moratalla et al., 2007). In garlic powder were detected high levels of tyramine and lower levels of phenylethylamine, but their contribution to the final

product contents in biogenic amines is not significant because they are added to sausage formulations in small quantities.

Table 4.9 ***Biogenic amine content evolution during fabrication process for the three batches (medium values ± standard deviation)***

Amines [mg/kg]	Days						
	0	*2*	*4*	*7*	*14*	*21*	*28*
Batch A							
Tryptamine	12,67±0,95	20,08±0,00	4,58±0,23	27,77±14,55	31,63±8,90	43,15±0,00	38,09±9,66
Phenyletilamine	ND	3,76±1,21	6,49±4,96	10,76±0,59	12,72±5,65	15,91±0,00	17,03±8,80
Putresceine	19,30±1,85	29,16±10,97	6,52±3,83	15,86±0,00	17,82±0,00	19,63±1,90	50,17±10,30
Cadaverine	8,38±1,78	41,67±6,98	39,95±0,00	80,56±50,43	81,20±31,56	25,07±1,17	84,34±11,88
Histamine	18,18±4,26	10,68±2,31	23,13±0,00	18,78±6,93	26,08±2,21	16,10±0,63	20,41±2,78
Tyramine	96,51±56,30	92,54±0,00	19,22±5,96	43,13±21,19	33,90±6,81	42,18±2,82	41,91±16,07
Spermidine	8,24±0,00	102,40±0,00	116,29±0,00	147,61±0,00	185,07±0,00	97,21±0,00	211,49±0,00
Spermine	15,03±3,04	14,30±2,79	8,82±2,61	14,27±2,01	15,50±1,24	13,88±2,87	15,06±2,11
Batch B							
Tryptamine	8,94±3,12	5,45±1,02	7,38±4,00	14,59±3,97	14,09±0,15	17,95±5,63	22,66±0,00
Phenyletilamine	3,13±0,00	3,57±0,00	3,17±0,00	2,93±0,00	12,45±4,93	14,20±0,00	6,22±0,00
Putresceine	16,97±0,00	32,97±16,35	19,98±8,48	38,82±14,42	11,92±2,63	36,61±11,45	43,38±0,00
Cadaverine	13,18±1,13	29,97±9,20	12,94±4,45	23,69±5,21	39,29±8,23	35,42±0,00	31,99±10,09
Histamine	4,91±0,66	7,68±0,53	15,93±1,34	9,86±1,71	12,24±0,50	20,43±2,42	18,58±5,28
Tyramine	47,48±8,74	41,66±10,67	49,78±0,00	30,56±12,46	38,48±29,31	25,49±0,00	21,68±11,86
Spermidine	ND	ND	ND	12,01±1,73	11,10±6,51	37,01±0,00	34,94±0,00
Spermine	14,03±1,19	14,28±3,34	8,34±1,86	13,59±3,10	13,21±1.53	11,34±1,19	13,58±2,89
Batch C							
Tryptamine	7,02±0,27	8,15±2,02	8,59±1,29	9,75±3,45	9,83±1,24	9,22±4,94	9,79±2,89
Phenyletilamine	29,37±0,00	24,06±10,21	7,18±0,57	15,82±9,80	4,09±0,43	25,51±10,93	10,31±0,00
Putresceine	3,05±0,22	6,36±2,67	11,56±8,79	22,26±10,67	13,78±0,37	16,68±9,85	25,05±15,94
Cadaverine	18,80±0,93	27,71±4,20	21,19±5,32	19,91±5,62	33,99±4,67	32,38±0,00	36,40±5,45
Histamine	7,47±4,53	14,06±8,15	25,92±12,49	22,73±11,93	13,43±6,90	14,97±0,00	15,07±8,31
Tyramine	51,49±15,23	65,48±9,93	66,16±6,15	48,98±7,27	18,91±0,00	35,48±0,00	34,86±0,00
Spermidine	ND	18,76±0,00	ND	13,42±0,00	17,58±5,69	22,36±0,00	28,32±0,00
Spermine	13,71±0,51	14,54±0,39	14,29±0,92	14,57±1,50	13,30±0,84	9,22±1,60	14,34±0,32

Phenylethylamine is always associated with high levels of tyramine, this relationship being attributed to the fact that microorganisms with strong tyrosine-decarboxylase activity also have moderate capacity to decarboxylate phenylalanine (Bover-Cid et al., 2001).

Spices might have been vehicles for potentially aminogenic microorganisms to meat batter or eventually amino acid-decarboxylase enzymes, especially pepper (Bover-Cid et al., 1999) and garlic powder (Latorre-Moratalla et al., 2007). In garlic powder were detected high levels of tyramine and lower levels of phenylethylamine, but their contribution to the final product contents in biogenic amines is not significant because they are added to sausage formulations in small quantities.

The final biogenic amine content was significantly lower ($p < 0,05$) in the inoculated batches C (174,14 mg/kg) and B (193,03 mg/kg), while in batch A we had 478,5 mg/kg at the end of 28 days.

We can conclude that starter cultures had a significant influence on biogenic amine content reduction in the three Dacia sausages produced.

Chapter 5
STUDY OF LYPOLITICAL PARAMETERS OF FAT. QUANTIFICATION OF TOTAL AND FREE FATTY ACIDS

5.1. Peroxide index

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. Figure 5.1. shows the evolution of this parameter during production process in the three Dacia batches.

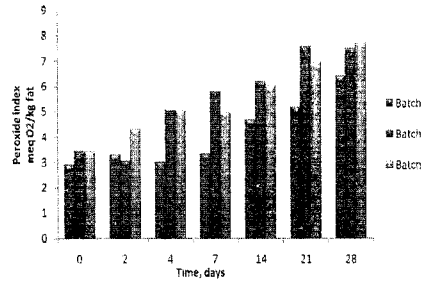

Figure 5.1. ***Peroxide index evolution during fabrication process***

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. The fact that the peroxide value increased from the 7^{th} day of fabrication, after fatty acids liberation, confirms the fact that peroxides are produced in dry sausages from the fatty acids liberated by lipolysis in the early stages of ripening.

These values are in the range described in the literature by Pearson et al. (1983) and Chasco et al. (1993).

5.2. Acidity index

Figure 5.2. shows the evolution of the acidity index of fat in the three Dacia batches during production process.

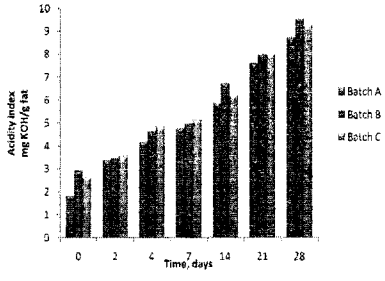

Figure 5.2. ***Acidity index during fabrication process***

The acidity index of fat showed no significant differences ($p > 0.05$) between batches during the entire process and a progressive liberation of fatty acids could be seen. The acidity index of fat increased from medium values of 2 mg KOH/100 g of dry matter (before filling) till final values of 9.5 mg KOH/100 g of dry matter. Our values were similar to those reported by Dominguez Fernández and Zumalacarregui Rodríguez (1991) in "chorizo de Leon" and Fernández-Fernández et al. (1997) in "Galician chorizo".

Lypolitical processes, specific for meat and meat fat tissue and those of microbial origin, leading to fatty acids liberation are the main causes for the acidity index increase during fabrication process (Lisazo et al., 1999).

Some authors consider that lypolisis intensity is variable not only between sausage type, but also between the replications of the same sausage (Dominguez Fernández and Zumalacárregui Rodríguez, 1991; Franco et al., 2002), the preparation method (Dominguez Fernández and Zumalacárregui Rodríguez, 1988; 1991) and raw materials (Lois et al., 1987).

This variability is not surprising if we take into consideration that fatty acids liberation is a phenomenon catalysed by tissue lipases (Garcia et al., 1992; Toldra, 1992) and microbial lipases (Nurmi and Niinivara, 1964; Cantoni et al., 1966b; Cantoni et al., 1967; Leistner and Ayres, 1967; Leistner and Bern, 1970; Langner, 1972; Demeyer et al., 1974;

Dobbertins et al., 1975; Comi and Cantoni, 1980; Smith and Alford, 1986) and their activity depends of different factors, like salt content and temperature (Motilva et al., 1992; 1993).

5.3. Total fatty acids content

Extraction of fat was performed according to Folch, Lees, and Stanley (1957). The values of acidity of the fat were determined using the Spanish Official Standard UNE 50.011. Free fatty acids were separated from the triglycerides in polypropylene columns packed with NH2-aminopropyl, following the procedure described by Antequera et al. (1992). The procedure described by Schlenk and Gellerman (1960) 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 lm 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); 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.

Results and discussion

Lypolisis has a direct influence in flavor compounds generation during ripening of dry fermented sausages.

In figures 5.4, 5.5 and 5.6 we can see the evolution of the most important fatty acids from total lipids structure during fabrication process of the three Dacia batches.

Different fatty acids percentages, from the three sausages glycerides and phospholipids, were constant throughout the entire fabrication process. The fatty acids of total lipids profile from sausage mix before filling, coincides with the one found by Franco et al. (2006), Martínez Suárez et al. (2007).

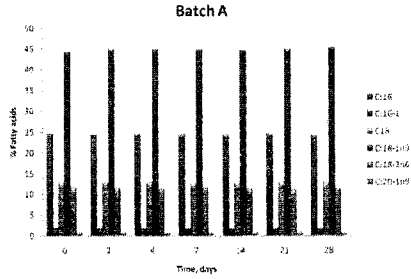

Figure 5.4. Total lipids most important fatty acids evolution during fabrication process

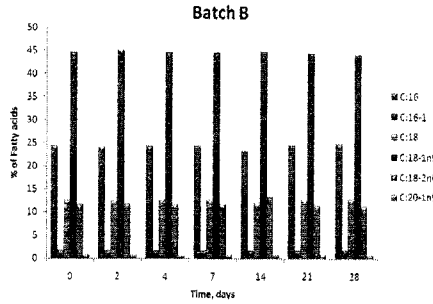

Figure 5.5. Total lipids most important fatty acids evolution during fabrication process

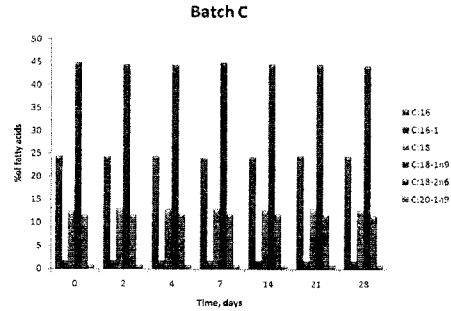

Figure 5.6. Total lipids most important fatty acids evolution during fabrication process

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 three batches had fatty acids percentages of about 38%. The most abundant were palmitic acid and stearic, with 24 % and 12 % from fatty acids total. Monounsaturated fatty acids, with 47 % were the most abundant, oleic acid representing 44 % from fatty acids total. Linoleic acid was the most important fatty acid from polyunsaturated ones reaching values around 14 % from fatty acids total.

Fat from pork meat is the most variable component, depending on pork degree of fattening, meat separation, anatomic parts taken into discussion. Anatomic localization of fat deposit affects texture and fat composition.

Fat deposits differ by fatty acids composition of lipids, fatty acids palmitoleic, oleic and stearic being the main contributors to differences between fat characteristics (Shorland, 1962; Kemp, 1981).

Many nutritional theories have focused their attention on the numerous health implications of the fatty acid profile of the diet, in particular the relationship between saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), long-chain polyunsaturated fatty acids (PUFA) of both the n-3 and n-6 categories, seems to play a major role.

As shown in numerous studies, coronary heart diseases are largely influenced by dietary habits in correlation with the characteristic lipid profile.

The dietary factors promoting and protecting against the onset of CHD are directly correlated to qualitative aspects of the lipid fraction in relationship to the percentage amount of some specific SFA, MUFA and PUFA of the series n-3 and n-6. To try to sum up these numerous effects, Ulbricht and Southgate (1991) proposed equations for the calculations of two indices, called by the authors the thrombogenicity index (IT) and the atherogenicity index (IA).

The equations proposed by the authors to calculate the indices of atherogenicity and thrombogenicity are:

$$IA = [(a*12{:}0) + (b*14{:}0) + (c*16{:}0)] \times [d*(AGPN\ n\text{-}6 + n\text{-}3) + e*(MUFA) + f*(MUFA - 18{:}1)] - 1$$

$$IT = [g*(14{:}0 + 16{:}0 + (18{:}0)] \times [(h*MUFA) + i*(MUFA - 18{:}1) + (m*n\text{-}6) + (n*n\text{-}3) + (n\text{-}3/n\text{-}6)] - 1$$

Where a,c,d,e,f = 1; b = 4; g = 1; h,i,m = 0.5 and n = 3.

These indices could be used as a tool to compare the health quality of the lipid fraction of different food.

The h/H ratio (between hipocholesterolic fatty acids and hipercholesterolic fatty acids), medium values found in our work being around 2.20, similar to those reported by Fernández et al. (2007) for *Jamon*.

$$h/H = [(C18:1 + C18:2n\text{-}6 + C18:3n\text{-}6 + C20:3) / (C14 + C16)]$$

The atherogenicity index calculated had values between 0.43 - 0.46 in the three batches, those levels decreasing at the end of drying-ripening period to 0.34 for batch A, 0.33 for batch B and 0.31 for batch C.

For thrombogenicity index values were between 1.02 – 1.16 at the beginning of production process, those values decreasing at the end to 0.78 for batch A, 0.75 for batch B and 0.73 for batch C.

5.4. Free fatty acids content

Levels of free fatty acids (expressed as mg/100 g of fat) increased significantly throughout the fabrication process in the three batches, reaching final values of : 1663.13 mg/100 g of fat in batch A, 1604.17 mg/100 g of fat in batch B and 1612.36 mg/100 mg of fat in batch C.

In the same time the content in each fatty acid in all types of sausages. In the drying-ripening period the major increase may be observed in the values of unsaturated fatty acids, which increase was of 7 - 9 times.

Values obtained in our work for each free fatty acid identified and quantified for each batch are presented in tables 5.1, 5.2 and 5.3.

From the distribution of the principal groups of fatty acids, we can see that the three batches are not significantly different ($p > 0,05$).

Taking into consideration the unsaturation degree of fatty acids, we can see that the unsaturated fatty acids (UFA), the monounsaturated ones respectively, were the most abundant, followed by the saturated and polyunsaturated fatty acids. As we know a ratio between saturated and monounsaturated fatty acids, with a greater amount of monounsaturated fatty acids brings benefits to humans health (Livesey, 2000; Gambacorta, 2009). The low polyunsaturated fatty acids content, makes this product less susceptible to oxidative changes, but is not contributing in a decisive manner to the essential fatty acids input.

*Table **Error! No text of specified style in document.**.1 Free fatty acids (mg/100g of fat)in batch A (control) during fabrication process (medium values ± standard desviation)*

Fatty acid (mg/100g of fat)	Days						
	0	2	4	7	14	21	28
C10:0	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	0,26±0,07	0,29±0,05	0,27±0,04	0,39±0,11	0,66±0,31	1,02±0,48	1,20±0,36
C14:0	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	0,13±0,03	0,19±0,03	0,23±0,01
C15:0	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
C16:0	63,23±15,30	95,05±38,29	75,89±3,88	124,26±41,91	171,71±75,47	253,23±105,14	307,88±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
C17:0	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±1,40
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
C18:0	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	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	0,096±0,05	0,062±0,05	0,11±0,00	0,21±0,05	0,33±0,06	0,35±0,06
C22:0	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

Fatty acid (mg/100g of fat)	Days 0	2	4	7	14	21	28
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	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	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
SFA	101,51±21,27	148,74±56,34	124,45±4,62	204,53±62,78	274,65±101,67	406,77±144,31	499,08±140,09
UFA	163,95±32,28	254,64±63,89	245,15±14,00	415,69±128,80	613,63±195,05	938,70±237,80	1164,04±272,33
MUFA	117,81±20,54	183,55±49,47	170,73±8,47	287,47±92,99	421,30±136,94	632,76±189,16	802,93±193,00
PUFA	46,13±12,26	71,09±14,42	74,42±8,22	128,21±36,35	192,33±61,20	305,94±57,15	361,11±85,58
∑n-3	2,77±0,77	4,18±0,96	4,58±0,58	8,16±2,45	13,09±4,44	21,57±4,94	25,91±5,43
∑n-6	40,20±10,40	60,67±16,81	63,71±4,30	112,86±37,46	165,65±51,48	255,49±48,22	307,58±69,56
PUFA/SFA	0,45±0,06	0,49±0,08	0,59±0,05	0,63±0,07	0,71±0,18	0,78±0,18	0,73±0,14
IA	0,46±0,04	0,44±0,05	0,38±0,03	0,37±0,02	0,35±0,07	0,34±0,07	0,34±0,05
IT	1,16±0,07	1,07±0,13	0,93±0,07	0,88±0,06	0,81±0,12	0,78±0,12	0,78±0,10
h/H	2,21±0,16	2,34±0,28	2,70±0,24	2,82±0,16	3,06±0,58	3,16±0,53	3,14±0,44

Table 4.2 ***Free fatty acids (mg/100 g of fat) in batch B during fabrication process (medium values ± standard desviation)***

Fatty acid (mg/100g of fat)	*Days*						
	0	*2*	*4*	*7*	*14*	*21*	*28*
C10:0	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	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
C14:0	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	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
C16:0	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±53,79
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
C17:0	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
C18:0	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	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	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	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

Fatty acid (mg/100g of fat)	Days 0	2	4	7	14	21	28
C24:0	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
SFA	68,94±19,78	154,03±65,91	180,69±13,30	283,73±100,91	317,27±118,37	406,63±103,88	476,58±62,97
UFA	123,06±30,37	242,40±92,05	331,575±65,19	600,54±267,94	720,09±242,53	979,12±224,75	1127,59±135,32
MUFA	87,64±27,72	167,96±60,77	229,67±45,77	393,37±161,93	481,80±178,21	655,36±178,21	765,13±106,82
PUFA	35,42±5,30	74,43±31,64	101,90±23,25	258,36±82,22	273,39±67,46	323,75±55,03	362,45±29,27
∑n-3	2,32±0,49	4,08±1,45	6,12±1,30	23,21±16,06	15,93±4,26	21,83±3,95	25,46±1,79
∑n-6	29,70±3,99	59,25±20,86	84,98±13,59	168,06±98,10	201,498±56,05	279,25±44,59	310,18±27,63
PUFA/SFA	0,53±0,13	0,48±0,06	0,56±0,10	0,70±0,13	0,76±0,12	0,81±0,13	0,76±0,04
IA	0,43±0,04	0,44±0,03	0,40±0,04	0,35±0,05	0,32±0,03	0,32±0,04	0,33±0,03
IT	1,02±0,08	1,10±0,06	1,02±0,11	0,80±0,11	0,77±0,05	0,75±0,04	0,75±0,02
h/H	2,39±0,25	2,34±0,14	2,54±0,28	3,00±0,49	3,25±0,30	3,26±0,30	3,19±0,24

Table 4.3 ***Free fatty acids (mg/100 g of fat) in batch C during fabrication process (medium values ± standard desviation)***

Fatty acid (mg/100g of fat)	*Days*						
	0	*2*	*4*	*7*	*14*	*21*	*28*
C10:0	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	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
C14:0	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	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
C16:0	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
C17:0	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
C18:0	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	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	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	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

Fatty acid (mg/100g of fat)	Days 0	2	4	7	14	21	28
C24:0	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
SFA	81,15±31,13	130,14±39,14	207,94±57,31	205,03±76,47	271,88±121,42	406,92±131,29	600,87±268,44
UFA	137,24±47,49	209,41±66,79	320,29±100,24	343,14±248,53	625,24±352,65	953,49±351,03	1459,63±630,36
MUFA	97,36±35,94	151,37±44,98	182,33±121,35	213,37±191,85	417,44±260,27	624,629±225,71	978,47±472,87
PUFA	39,87±11,59	58,03±22,10	137,95±54,53	129,76±62,69	207,80±92,40	328,86±125,34	481,16±158,02
∑n-3	2,59±0,42	3,10±1,26	8,84±4,41	8,63±4,84	13,38±6,46	21,05±6,32	32,07±10,44
∑n-6	32,79±8,66	45,97±15,52	118,60±55,30	108,13±48,94	172,09±78,30	271,04±86,49	406,15±141,95
PUFA/SFA	0,51±0,10	0,44±0,04	0,65±0,10	0,61±0,07	0,76±0,00	0,80±0,06	0,82±0,11
IA	0,45±0,04	0,46±0,00	0,50±0,21	0,56±0,28	0,35±0,04	0,33±0,02	0,31±0,04
IT	1,06±0,20	1,14±0,05	1,15±0,32	1,30±0,65	0,81±0,07	0,76±0,02	0,73±0,07
h/H	2,26±0,19	2,20±0,05	2,19±0,87	2,10±0,98	2,99±0,39	3,23±0,18	3,36±0,41

Legend:

SFA: sum of saturated fatty acids;

UFA: sum of unsaturated fatty acids;

MUFA: sum of monounsaturated fatty acids;

PUFA: sum of polyunsaturated fatty acids;

∑n-3: sum of fatty acids n-3;

∑n-6: sum of fatty acids n-6;

PUFA/AGS: polyunsaturated and saturated fatty acids ratio;

IA: atherogenicity index;

IT: thrombogenicity index;

h/H: hipocolesterolemic and hipercolesterolemic fatty acids ratio.

Each fatty acid increased during fabrication process. Significant increases during drying-ripening were observed in the case of oleic, linoleic, palmitic and stearic fatty acids, which confirms the data reported by other authors (Hernández et al., 1999; Gambacorta et al., 2009).

Kenneally, Schwarz, Fransen and Arendt (1998) reported that there were no differences between the batches inoculated with lypolitical starter cultures and those inoculated with non lypolitical starters. Differences were observed between replications, probably because of the differences in raw materials, which have an important influence on lypolisis.

Moltiva and Toldrá (1993) showed that the additives and technological conditions promote lipase activity by decreasing water activity and increasing salt concentration.

We can say that the lypolitical activity in the three Dacia batches is generated by tissue lipases and not by the added starter cultures, our results being in agreement with those reported by other authors (Molly et al., 1996; Zanardi et al., 2004).

Our final values are situated in the range described in the literature for other dry sausages, free fatty acids representing between 1 to 7 % of total fat (Johanson et al., 1994; Bolumar et al., 2001).

5.5. Conclusions for the physicochemical analysis

Moisture decreased progressively during the entire fabrication process, being the same for the three batches because of the same caliber of sausages, the same ripening conditions and the same salt content.

Water activity is a extremely important parameter, from a microbiological point of view and for dry sausages stability. During the ripening stage, this parameter had a similar decreasing evolution for the three batches, leading to the inactivation of spoilage bacteria.

The pH evolution was also similar for the three batches, values decreasing progressively till the 14 day of drying-ripening, then values had a slow increase till the end of 28 days. The values obtained for this parameter prove the benefits of starter cultures on the acidification process and on the spoilage bacteria inhibition.

Salt content, expressed as g/100 g of dry matter had a constant evolution during the fabrication process, the same trend being observed for the protein and lipid content.

The titratable acidity had a reverse evolution compared with the pH, increasing rapidly in the first 7 days, then slowly till the 14 day, then a slow decrease being observed till the end of the process.

Nitrate conversion to nitrite and the specific color formation was a continuous process, having an increased rate in the first four days.

The most important biogenic amine, form a quantitative point of view, was tiramine, followed by cadaverine and putresceine.

The total biogenic amine content was significantly lower ($p < 0.05$) in batch C, followed by batch B and then batch A. We can say that starter cultures utilization had a significant role in reducing the biogenic amine content.

The starter cultures had no significant effect ($p > 0.05$) in fat lypolisis, during the ripening process, confirming the role of the endogenous lipases in the changes at the lipidic system level in this technological step.

The increase of the peroxide index in the drying-ripening step was obvious because of the oxygen availability and in the same time with fatty acids liberation, which confirms the fact that the peroxides are produced in dry sausages from the fatty acids released during lypolisis.

Monounsaturated fatty acids were the most abundant, followed by the saturated and the polyunsaturated fatty acids.

Chapter 6
VOLATILE COMPOUNDS PROFILE. SENSORY ANALYSIS

6.1. Volatile compounds evolution

Analysis of volatile compounds

Samples were lyophilized (Labconco Corp., Freezone, Kans., U.S.A.) during 48 h at −40 °C and then samples were ground in a domestic blender, and 10 g weighed, put into a dynamic headspace vial. The volatile compounds were extracted and concentrated in a purge-and-trap concentrator coupled with a cryofocusing module (Teledyne Tekmar, Mason, Ohio, U.S.A.).

Dynamic headspace volatile concentration

Lyophilized samples were transferred into headspaced vials and concentrated in a purge-and-trap concentrator (Stratum, Teledyne Tekmar,Mason) equipped with a cryofocusing module was connected to an autosampler (Solatek 72 Multimatrix Vial Autosampler, Teledyne Tekmar, Mason). The sample was maintained at 80 °C for 1 min and then flushed with helium at a flow rate of 40 mL/min for 22 min. The volatile compounds were adsorbed on a Tenax Trap (Supelco, Bellafonte, Pa., U.S.A.). Volatile compounds were thermally desorbed from the Tenax trap at 225 °C for 4 min with a helium flow rate of 300 mL/min. The desorbed compounds were cryofocused at −30 °C using liquid nitrogen at the entrance of a DB-624 capillary column (J&W Scientific, Folsom, Calif., U.S.A.).

Gas chromatography/mass spectrometry

A gas chromatograph 6890N (Agilent Technologies Spain, S.L., Madrid, Spain) equipped with mass detector 5973N (Agilent Technologies Spain, S.L.) was used with a DB-624 capillary column (J&W scientific: 30 m × 0.25 mm i.d., 1.4-μm film thickness). The sample was injected in split mode (1:20). Helium was used as a carrier gas with a linear velocity of 36 cm/s. The temperature program used was as follows: 40 °C maintained for 2 min and then raised from 40 to 100 °C at 3 °C/min, then from 100 to 180 °C at 5 °C/min, and from 180 to 250 °C at 9 °C/min with a final holding time of 5 min; total run time 50.78 min. Injector and detector temperatures were set at 220 and 260 °C, respectively. The mass spectra were obtained using the mass selective

detector by electronic impact at 70 eV, a multiplier voltage of 1576 V, and collecting data at a rate of 6.34 scans/s over the m/z 40 to 300. Compounds were identified by comparing their mass spectra with those contained in the NIST05 (Natl. Inst. of Standards and Technology, Gaithersburg, Md.,U.S.A.) library and by matching their retention indices with those reported in literature. Nine samples were analyzed in triplicate. Results were reported as relative abundance expressed as total area counts (AU × 106).

Results and discussion

Several compounds were absent or in very low quantities in the initial mass, because strong lipolytical activities began after four days of ripening. Long and medium chain fatty acids were detected in high quantities in the three batches. These fatty acids may act as a source of compounds with certain effect on the aroma, but they are not directly involved in dry cured products aroma (Árboles and Juliá, 1992).

Although oleic acid was the main acid in all the three batches, acetic acid was the only acid determined in the three batches. The levels of acetic acid, found in this study, were about 25% in batch A, 19% in batch B and 10% in batch C from the total area of peaks. Presence of acetic acid was also reported by other authors (Berger et al,., 1990; Schmidt and Berger, 1998),in sausages, while Meynier et al. (1999) didn't detect it.

In general, with regard to volatile compounds derived from lipid oxidation, we can see that aldehydes were present in higher amounts in the inoculated batches than in control sausage. Aldehydes are probably the most interesting class of volatile compounds from flavor and odour generating point of view. This compounds represented 5% of the total peak area at the end of process. The main aldehyde was hexanal, which offers a green grass odour (Stahnke, 1994), being produced during the oxidative degradation of unsaturated fatty acids. High concentrations of hexanal signal flavor deterioration in meat products often resulting in a rancid aroma (Pham et al., 2008; Ramirez and Cava, 2007). In addition, heptanal and butanal were also detected. There was one branched short-chain aldehyde 3-methyl-butanal, which is produced during the degradation of leucine through a non enzymatic Strecker reaction (Berdague et al., 1993) or by microorganisms. This compound was associated with a ripened aroma in cured meat products (Søndergaard and Stahnke, 2002). It can be transformed, into its corresponding alcohol, acid and even ester, as it is of great importance in the final flavor of the products.

Alcohols are mainly generated as reaction products of lipid oxidation (Shahidi, Rubin & D'Souza, 1986). In this study, two alcohols were identified, but only one, 1-pentanol, appeared in one of the inoculated sausages till the end of ripening period. 1-pentanol was also detected by Garcia-Esteban, Ansorena, Astiasaran, and Ruiz (2004), Muriel et al. (2004) and Ramirez and Cava (2007) in other raw cured meat products. In general, primary unbranched alcohols produce grassy or woody aromas and make an overall contribution to the odour (Garcia et al., 1992). Because of their low odour threshold they are important contributors to the aroma of these products (Sabio et al., 1998).

The alkanes were found in small amounts and their origin is probably in the oxidation of branched fatty acids present in animal tissues or from the unsaponifiable fraction of vegetable feed of animals. Several hydrocarbons have been identified in the volatile fraction of the dry sausages produced. Aliphatic hydrocarbons increased during processing but the percentage of these compounds in the total chromatographic area decreased as processing time elapsed. Aliphatic hydrocarbons do not contribute significantly to the aroma of dry-cured meat products because of their high threshold value (Ramirez and Cava, 2007). The percentage of these compounds was significantly higher in batch B. Low weight hydrocarbons, such as octane, hexane and heptanes, were found in all the batches.

The same situation was found for the ketones, being found in small amounts and deriving from lipid oxidation. Three ketones were identified, 3-hexanone, 2-cyclopenten-1-one, 2-methyl and 2,3-butandione. Diacetyl imparts a buttery odour, having a characteristic sweet odour and a low sensory threshold and, according to Stahnke (1995), is of great importance to the final aroma.

Furans weren't detected in sausages matrix before filling, the only compound detected after four days being 3-methylfuran.

Esters have been indicated as important volatiles in fermented sausages (Edwards et al., 1999; Stahnke, 1994) and they are also present in high quantities in our study. They originate from alcohols and carboxylic acids by the action of microorganisms (Sabio et al., 1998). They represented about 47% in control batch and in batch B, reaching 61% in batch C (Fig.1).

It is well documented that many strains of lactic acid bacteria used as starter cultures are able to produce esters (Hosono et al., 1974; Liu et al., 1998). They have low odour threshold values and impart fruity notes (Stahnke, 1994), being associated, together with branched aldehydes, to ripened flavor in cured meat products (Hierro et al., 2004). Methyl-esters were the

main esters produced in the three batches and their production may be attributed to microorganisms if we take into consideration the high microbial counts in the present study (~11 log c.f.u). the main ester identified in this study was the metylic ester of acetic acid, which represented about 40% from the total peack area.

Table 4.1. ***Volatile compounds evolution during the fabrication process (medium values ± standard deviation)***

	Batch A (days)				*Batch B(days)*				*Batch C (days)*				
	7	*14*	*28*	*D*	*7*	*14*	*28*	*D*	*7*	*14*	*28*	*D*	*B*
Compounds % from the total area of peacks													
Terpenes													
alpha pinene	ND	ND	0.92±0.15	***	ND	0.46±0.46	0.84±0.22	*	1.05±0.10	0.54±0.28	1.17±0.20	-	-
beta pinene	ND	ND	0.59±0.29	***	ND	0.2±0.2	0.17±0.17	-	0.41±0.05	0.32±0.16	1.09±0.21	*	-
beta myrcene	ND	ND	0.44±.,05	***	ND	ND	0.18±0.18	-	ND	ND	0.44±0.24	-	-
alpha phellandrene	ND	0.15±0.15	ND	-	0.14±0.14	0.19±0.19	ND	-	ND	ND	0.14±0.14	-	-
3-carene	ND	ND	4.27±0.33	***	7.57±1.53	ND	3.76±1.20	**	7.90±0.88	ND	4.69±0.43	***	-
D-limonene	0.27±0.27	0.35±0.35	2.53±0.33	**	ND	ND	1.92±0.39	**	ND	ND	2.71±0.48	***	-
Caryophylene	ND	ND	ND	-	ND	ND	ND	-	ND	ND	ND	-	-
Total	**0.27±0,27**	**0.51±0.30**	**8.74±0.64**	***	**7.72±1.40**	**0.84±0.84**	**6.88±1.57**	*	**9.37±0.90**	**0.86±0.43**	**10.26±1.62**	**	-
Amines													
Methenamine	0.60±0.30	1.52±0.26	0.20±0.10	*	1.67±0.92	0.71±0.36	0.12±0.12	-	1.19±0.25	0.80±0.12	0.09±0.09	**	-
Furans													
furan.3-methyl	ND	ND	ND	-	0.29±0.29	0.99±0.72	0.85±0.46	-	13.44±2.74	1.01±0.71	0.57±0.57	**	-
	3.61±0.93	5.18±2.14	1.70±0.44	-	27.28±2.07	7.63±1.48	2.27±0.28	***	20.41±2.10	5.35±1.37	2.56±0.33	***	-
Aldehydes													
pentanal	ND	ND	ND	-	ND	ND	ND	-	0.20±0.20	ND	ND	-	-
hexanal	ND	ND	0.25±0.24	-	1.187±0.67	0.48±0.46	0.52±0.52	-	0.44±0.25	0.56±0.56	0.45±0.45	-	*
2-hexenal	ND	ND	0.13±0.13	-	ND	0.39±0.39	0.39±0.39	-	0.39±0.23	0.50±0.50	0.21±0.21	-	*
heptanal	ND	ND	ND	-	ND	ND	ND	-	0.19±0.19	ND	ND	-	-
2-heptenal	0.26±0.26	ND	0.13±0.13	-	ND	ND	0.29±0.29	-	ND	0.41±0.41	ND	-	-
nonanal	ND	ND	ND	-	ND	ND	ND	-	0.15±0.15	ND	ND	-	-
2-furancarboxaldehyde	0.84±0.42	ND	1.15±0.31	-	0.87±0.47	0.7±0.41	1.41±0.24	-	1.35±0.74	1.21±0.36	0.87±0.48	-	-
2.4-heptadienal	3.22±0.75	2.06±0.65	ND	*	3.24±0.24	2.42±0.25	0.89±0.45	**	3.41±0.63	3.39±0.77	0.89±0.18	*	-
butanal 3-methyl	ND	ND	ND	-	ND	ND	ND	-	ND	ND	ND	-	-

furfural	**8.53±1.69**	**8.76±2.23**	**3.57±0.84**	-	**34.54±2.29**	**13.31±2.50**	**6.74±2.25**	***	**41.21±4.12**	**13.27±1.74**	**5.68±1.51**	***	-
2-n-butylacrolein													
Total	ND	4.45±2.19	4.47±1.39	-	0.73±0.73	1.40±1.21	8.38±1.21	**	0.82±0.82	0.43±0.43	2.28±0.87	-	-
Hydrocarbons	ND	0.71±0.35	ND	-	ND	ND	ND	-	1.11±0.35	0.18±0.18	ND	*	-
Heptane, 2.2 4.6.6-pentamethyl	3.32±3.09	3.41±1.61	0.17±0.17	-	ND	ND	0.43±0.22	-	0.94±0.94	1.28±1.28	ND	-	-
Decane, 2.2.4-trimethyl	0.88±0.88	2.21±1.13	ND	-	1.08±0.13	ND	ND	***	1.24±0.26	1.13±0.06	ND	*	-
2.2.7.7- tetramethyloctane	ND	ND	ND	-	2.72±0.25	0.85±0.43	ND	**	3.06±0.63	2.69±0.34	ND	**	-
Nonane, 5-methyl	1.85±0.25	0.91±0.47	ND	-	1.89±0.35	1.26±0.63	ND	*	1.42±0.23	1.62±0.33	ND	*	-
Nonane, 3.7-dimethyl	1.27±0.22	1.69±0.84	ND	-	2.43±0.13	ND	ND	***	2.51±0.42	2.15±0.58	ND	*	-
	ND	ND	ND		ND	ND	ND		ND	ND	ND		
Undecane, 4.8-dimethyl				-				-				-	-
Undecane, 4-methyl	1.24±0.34	0.14±0.14	ND	*	0.44±0.28	ND	ND	-	ND	ND	ND	-	-
Heptane, 4-ethyl- 2.2.6.6-tetramethyl	0.23±0.23	0.15±0.15	0.33±0.33	-	0.77±0.32	0.52±0.52	0.64±0.64	-	1.03±0.58	0.10±0.10	ND	-	-
octane 2.6-dimethyl	ND	ND	0.12±0.12	-	0.61±0.47	ND	0.15±0.15	-	ND	0.39±0.22	0.29±0.29	-	-
octane	2.67±1.85	3.90±0.50	1.75±0.38	-	2.77±0.62	5.01±0.32	1.92±0.55	-	2.16±0.49	2.69±0.19	1.61±0.27	-	-
	ND	ND	0.79±0.16		ND	1.42±0.71	0.52±0.26		ND	ND	0.84±0.13		
heptane				***				-				***	-
hexane	**19.55±2.25**	**17.61±5.39**	**7.65±2.04**	*	**13.48±0.98**	**10.48±1.22**	**12.06±1.66**	-	**14.33±1.28**	**12.7±2.25**	**5.05±1.32**	*	-
benzene 1-methyl-4-(1-methylethyl)													-
Total	ND	ND	ND	-	0.29±0.29	ND	0.32±0.32	-	0.53±0.53	ND	ND	-	-
Alchools	ND	ND	ND	-	ND	ND	ND	-	ND	ND	ND	-	-
1-pentanol	**ND**	**ND**	**ND**	-	**0.29±0.29**	**0**	**0.32±0.32**	-	**0.53±0.53**	**ND**	**ND**	-	-
1-octanol, 2-butyl													
Total	0.06±0.06	ND	1.06±0.08	***	ND	0.23±0.23	0.93±0.14	*	ND	0.34±0.34	0.91±0.54	-	-
Ketones	2.67±1.39	3.21±0.54	1.04±0.88	-	2.96±2.00	1.5±0.80	0.43±0.43	-	2.88±1.28	2.17±0.70	0.33±0.23	-	-
2-cyclopenten-1-one. 2-	ND	ND	1.44±0.97	-	ND	ND	ND	-	ND	ND	ND	-	-

methyl													
2.3-butanedione	**2.67±1.39**	**3.21±0.54**	**2.49±1.24**	-	**2.96±2.00**	**1.5±0.80**	**0.43±0.43**	-	**2.88±1.28**	**2.17±0.70**	**0.33±0.23**	-	-
3-hexanone													
Total	ND	ND	28.78±3.12	***	15.11±1.41	43.38±4.81	19.04±1.10	**	24.55±4.53	36.99±4.12	10.43±2.45	**	-
Acids													
Acetic acid	27.18±3.61	32.99±4.02	31.11±3.61	-	23.11±1.26	31.24±4.62	33.86±5.71	-	7.74±1.55	28.80±2.46	40.78±2.87	***	-
Esters	1.06±0.64	0.40±0.40	5.28±0.74	**	0.20±0.20	0.73±0.38	6.59±0.83	***	0.30±0.30	0.76±0.38	13.17±1.24	***	-
Acetic acid, methyl ester	ND	ND	1.29±0.23	***	ND	ND	0.50±0.26	-	ND	ND	3.39±1.04	*	-
butanoic acid, methyl ester	0.17±0.17	0.14±0.14	2.07±1.15	-	0.46±0.46	0.38±0.19	1.75±0.24	**	0.82±0.82	0.58±0.32	2.99±0.81	-	-
pentanoic acid, methyl ester	ND	ND	0.28±0.28	-	ND	ND	ND	-	ND	ND	0.62±0.33	-	-
hexanoic acid, methyl ester	1.04±0.33	0.51±0.05	0.24±0.13	-	0.25±0.13	0.15±0.15	0.20±0.20	-	0.25±0.04	0.24±0.12	ND	-	-
Octanoic acid. methyl ester	2.73±0.69	1.75±0.16	1.69±0.53	-	0.81±0.15	ND	1.25±0.17	**	0.66±0.07	0.96±0.13	1.43±0.62	-	-
Malonic acid. bis (2-trimethylsilyl ethyl ester)	ND	0.51±0.26	5.45±1.38	**	1.02±0.31	0.37±0.19	4.43±0.44	***	1.37±0.35	1.20±0.11	2.56±0.50	-	-
Total	**32.21±4.56**	**36.34±4.65**	**47.45±0.77**	-	**25.87±0.99**	**32.50±5.50**	**48.61±6.94**	-	**11.16±0.66**	**32.56±2.46**	**64.98±5.49**	***	-

D: significantly different values as influenced by time; B: significantly different values as influenced by starter culture.

***P<0.001; **P<0.01; *P<0.05; - no significant difference

ND: no detected

Terpenes represented between 8 and 10% of the total peak area, having their origin in the use of spices in sausages. Some of them were described as fruity, floral and fresh rather than spicy (Meynier et al., 1999). In dry-cured ham, it is suggested by Sabio et al. (1998) that the presence of limonene is associated with the pig diet. In this study, the most important terpenes found were 3-carene and D-limonene. This two products, as the other five in this work, are thought to have their origin in the use of black pepper. α-phelandrene, β-mircene and cariophilene have their origin in the use of black pepper.

6.2 Sensory analysis

The sausages were submitted to sensory evaluation to ascertain if there are differences between control sample and samples inoculated with starter cultures. The sensory quality of fermented dry sausages produced with different starter cultures was evaluated by a panel of seven trained assessors, professors, researchers and PhD students at Vigo University, Faculty of Sciences, Ourense, Spain. They were trained through three preliminary sessions, in order to familiarize them with samples under investigation. Finally 20 attributes were selected, using a 1(very low perception or absence) to 10 (very intense perception) scale. Descriptors were established during the first three sessions and included: 5 appearance, 7 section appearance, 7 mastication, 1 general perception attributes (Table 1). We used 0,5 cm thick slices, cut with a knife and served at room temperature on white plastic dishes. Water and unsalted crackers were provided to cleanse palate between samples.

Table 6.2. Sausage descriptive attributes and definitions

Attribute	**Definition**
Appearance	
Appearance	External impression
Hardness	Strenght requiered to deform the sausage
Smell intensity	Intensity of smell when the sausage isn't cut
Smell quality	Specificity of the smell for this kind of product
Overall external perception	
Section appearance	

Fat/lean demarcation	The degree of distinction between fat and meat
Cohesivnes	Deformation degree before breaking the slice
Easy peeling capability	The work requiered to peel of the membrane
Intensity of smell	The intensity of smell at slicing
Smell quality	Specificity of the smell for this kind of product
Colour intensity	Red colour intensity
Overall perception in section	Proportion of fat particles over lean meat in the slice
Mastication	
Flavour intensity	Intensity of flavour at mastication
Flavour quality	Associacion of this flavour with this tipe of product
Chewiness	The work requiered to masticate before swallowing
Juiciness	Perception of the amount of water relesed by the product
Salty	Flavour associated with salt
Smoky	Flavour associated with smoke
Overall perception at mastication	General perception after mastication and swallowing
Overall acceptability	

From figure 6.2 it can be seen that the three dry sausages had the same scores for the visual appearance. For the hardness, intensity and quality of smell the inoculated batches had higher scores ($p < 0.05$) than the control batch.

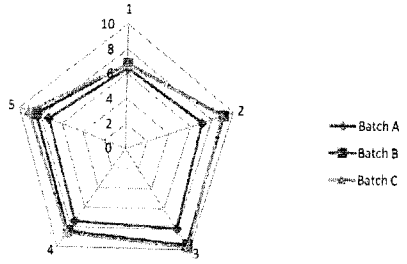

Figure 6.2. External evaluation of Dacia sausages

In figure 6.3. we can see that the attributes used to describe the three sausages had the same evolution for almost all the attributes. Only smell intensity and quality had higher scores for the inoculated batches.

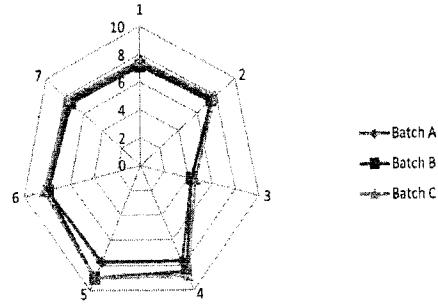

Figure 6.3. Section analysis of the three batches

We can see in figure 6.4 the evaluation of Dacia sausages after mastication. Tensity Higher scores were obtained for the taste intensity and the quality of taste in the inoculated batches, compared to control.

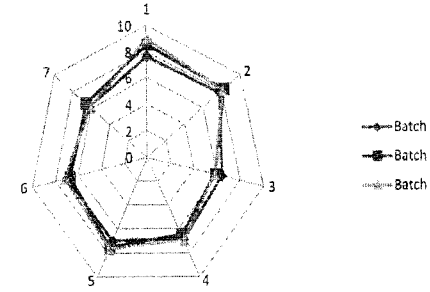

Figure 6.4. Evaluation of Dacia sausages after mastication1

6.3. Conclusions

The starter cultures used in this work contributed to the dry fermented sausages aroma and their contribution was strain combination dependent.

Volatile compounds profiles in the three batches were statisticaly different ($p < 0.05$), with type, number and activity of starters as main factors influencing this differences.

The sensory analysis showed differences only between control and the inoculated batches, the last ones having similar results for the sensory atributes taken into consideration. The starter cultures had a greater influence on the flavour intensity and quality.

Chapter 7
FINAL CONCLUSIONS

The obtained dry fermented sausages, are part of the category of dry meat products, their stability being achieved by low values of pH and water activity, low moisture content and dominance of lactic acid bacteria.

The final product characteristics were the result of a complex of physicochemical, biochemical and microbiological desired processes with a role in the formation, improving and equilibration of the physicochemical and sensory characteristics and in product conservation.

The association between strains of *Lactobacillus sakei* and *Staphylococcus equorum* or *Lactobacillus sakei, Lactobacillus acidophilus* and *Staphylococcus equorum* lead to a domination of lactic acid bacteria during the entire production process.

Lactic acid bacteria, by their fermentative metabolism, determined the proper acidification of the sausages, acceleration of the drying process, texture formation and created inadequate medium conditions for spoilage and pathogenic bacteria.

The pH values were significantly lower ($p < 0.05$) in the inoculated batches, around 5.20, compared to the control were this parameter reached 5.42 at the end of 28 days. This fact points out the benefic effect of starter cultures in the acidification process.

Evaporation process lead to low contents in water and a concentration of protein, lipids and salt contents, without any significant differences ($p > 0.05$) between the three batches. The values obtained for those parameters were in the range reported by other authors for this kind of products.

Final values of water activity were 0.820-0.830 and final moisture content was about 30%. These values prove that our products are in the dry sausages class.

Nitrate conversion to nitrite, followed by its degradation to NO assured for the three batches a conversion percent of myoglobin to nitrozomioglobine, sufficiently high to ensure a specific pleasant color of the sausages.

Starter cultures had a positive effect on reducing the biogenic amine content in the inoculated batches. Biogenic amine levels were significantly different ($p < 0.05$) compared to

control and we can say that use of starter cultures appears like a necessity in dry sausage production to prevent/reduce the biogenic amine production.

The selected starter cultures didn't had important lypolitical activities and had no decisive effect on the lipid oxidation processes in the three sausages.

From a quantitative point of view, the fatty acids distribution had the following structure: monounsaturated fatty acids > saturated > polyunsaturated. The monounsaturated acids abundance is important from a nutritional point of view, while the lower content in polyunsaturated acids makes sausages less susceptible to oxidative changes, which can affect the sausage quality.

The oleic acid was the most abundant monounsaturated fatty acid, palmitic and stearic acids were quantitatively significant, while linoleic acid was the most abundant polyunsaturated fatty acid.

Starter cultures didn't had a significant effect in lypolisis and fatty acids distribution in the three Dacia sausages.

The volatile profiles of the three sausages had significant differences ($p < 0.05$), the responsible factors being the microorganisms type, number and activity.

The major volatile compounds of Dacia sausages, were metabolism products of the natural flora present in the sausages and of the added starters. Esters were the main group detected in the sausages, followed by acids, aldehydes, hydrocarbons and terpenes.

The sensory analysis pointed out significant differences ($p < 0.05$) between control and inoculated batches, with respect to flavor intensity and quality.

The starters used in this work had an important contribution to the dry sausages flavor, this depending on the microbial combinations used. This combinations proved to be efficient in dry sausages production with short ripening, from the product safety and standardization point of view.

The proposed production technology of Dacia sausages with starter inoculation ensures: reduction of the production period from 60 to 28 days, reduction of smoking period from 5-10 days to 3 hours (in an intensive system) and improvement of the sensory properties and consumers acceptability of the final products.

In the same time this work realized for the first time in a Romanian dry sausage the monitoring throughout the entire production process of total and free fatty acids liberation, the biogenic amines content and volatile compounds profiles.

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