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Dry sausages ripening: influence of thermohygrometric conditions on microbiological, chemical and physico-chemical characteristics

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Abstract

The DRIP (dry sausages ripening improved project) programme, financed by the EU, had the purpose of carrying out research into systems of improving the techniques for preparing matured salamis, special attention being paid to the phases of drying and ripening. With a view to arriving at solutions of use to operators the programme concerned the various aspects of production, with particular emphasis on the various phases of preparation, resulting in mathematical models able to describe the processes of heat and mass transfer and in the setting up of instruments designed to optimise the planning of ripening plants. The project will come to an end in the first few months of the year 2000; up to now, interesting results have been obtained regarding the evolution of the physicochemical parameters and the influence of the drying characteristics on the evolution of the various descriptors; the validation of the mathematical models is still in progress © 2000 Published by Elsevier Science Ltd. All rights reserved.

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1. Introduction

The salami is undoubtedly one of the first methods produced by man to conserve meat, long before he began to butcher animals. In many European countries, the lean and fat content of meat are seasoned with salt and other condiments and minced, then mixed with other substances characteristic of the region, and encased in a wrapping, originally the gut of the animal itself. These products have always been subjected to differing processes of fermentation/drying, for varying lengths of time at room temperature and under ventilation conditions designed to allow for a weight loss guaranteed to ensure adequate preservation.

The characteristics of salamis being thus closely dependent on the thermohygrometric conditions in the various regions, on the ingredients used and on the skills of the various populations, they are bound to differ greatly from region to region in composition, nutritional value and flavour.

The regions of Europe which look out onto the Mediterranean have products with similar characteristics: not too acid in flavour, generally not smoked and with aromas and flavours typical of varying ripening times.

The turn of the century witnessed a gradual changeover in practically all European countries from family to local small-scale and industrial production, that of salami included; only more recently has our knowledge widened concerning the mechanisms responsible for food conservation and for the formation of its organoleptic qualities, dating from the years immediately following the second world war. The studies on the latter aspects are still far from complete. Besides, most studies have been on specific aspects, very few works dealing with a combination of several aspects (microbiological and biochemical transformations, drying mechanisms and the diffusion of salt and water) (Campbell-Platt, & Cook, 1995).

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The means to obtain the necessary water loss and the consequent decrease in Aw have considerably changed in the last few decades, passing from systems based on the use of outside air to air-conditioned plants regulated according to criteria of varying degrees of sophistication. Plant design has not always been based on general optimisation criteria, but has favoured above all certain simple parameters such as weight loss, uniformity of treatment and energy saving.

In the preparation of salamis, two sets of parameters are fundamental — the composition of the mixtures and the drying methods; the first of these aspects has doubtless been the subject of a greater number of research studies, whereas drying methods have not been sufficiently studied at scientific level, the management of plants still being based essentially on the experience of the operators.

In order to address the aspects still left to be studied and to tackle the various problems in a co-ordinated manner, a DRIP (dry sausages ripening improved project) research project has been proposed, under the aegis of the fourth framework programme of the European Union, lasting 42 months and finishing in March 2000.

The project involves the participation of research associations (5 partners), salami-producing companies (3 partners) and manufacturers of conditioning plants for the ripening of meat products (2 partners).

The programme is organised around tasks enabling the various themes to be addressed in a logical and consequential way; at the outset, the aim of the study was to promote knowledge of the chemical, physicochemical and microbiological characteristics of salamis, and of the parameters directly connected to water loss (rate of evaporation and of diffusion) during the various phases of industrial preparation. Subsequently, a series of experiments on various drying and ripening methods was included, with the aim of setting up mathematical models able to describe the drying of products and the circulation of air inside the plant. To reach this latter objective, two pilot cells have been designed and built of the type based on the circulation and uptake of air from the ceiling, with a capacity of 10 m^3 and equipped with instruments able to modify the technological parameters (air velocity, temperature of the refrigerating liquid, ventilation times etc.) and to record in continuum the main thermohygrometric parameters [Temperatures (T) and relative humidity (RH) in different positions].

The cells were installed in two research centres, in Italy and in France, and were programmed for the ripening of three batches of each type of salami, the same as those analysed in the first part of the study, prepared at the factories taking part in the programme. During the ripening process, the products were subjected to various types of controls (weight loss, physicochemical, chemical and microbiological analyses and sensory evaluations).

At the time of going to press, the project is not yet finished, since the final series of processing at the pilot plant, designed above all for the validation of the mathematical models, is still in progress; essentially for this reason, this report will not deal with those aspects connected with the mathematical models, but will examine the results obtained so far regarding the physicochemical and microbiological parameters and industrial and pilot plant production, and in particular the variations in the chemical, physicochemical and microbiological descriptors connected with the various drying methods.

2. Materials and method.

The wide variety of products normally prepared in the various European countries meant that it was important to make precise product choices answering to rational requirements; thus, 6 types of salami were selected (two for each country) (Table 1), differing in size and average ripening times (a small salami of rapid fermentation and a bigger one with longer ripening times); this choice was made essentially for technological reasons, since the rate of drying and of Aw decrease in the internal fraction, factors which radically influence the quality of the finished product, basically depend on the distance of the centre of the salami from the surface.

2.1. Microbiological determinations

2.1.1. Meat starter and contaminant biota

Enumeration and identification of lactic acid bacteria (LAB): in MRS agar (Oxoid CM 361) acidified to pH 5.4 at 25°C. The plates were incubated at 37°C for 3 days in an anaerobic culture jar.

Micrococcaceae enumeration: Mannitol –salt agar (MSA, Oxoid CM 85 incubated at 30°C for 3 days. Colonies on MSA were examinatd for cel morphology, Gram reaction and catalase. The identification was carried out with the API Staph System.

Enterobacteriaceae enumeration: on violet-red bile glucose agar (VRBG Oxoid CM 485) incubated at 35-37°C for 24 h. Colonies surrounded by a purple zone were considered to be Enterobacteriaceae.

Enterococci enumeration : using Slanetz and Bartley medium (oxoid CM 3767) at 37°C for 48 h. The API 20 Strep System was utilised for identification.

Coliforms enumeration and *E. coli* identification: Coli ID (Biomerieux 42017) medium at 37°C for 24–48h.

2.1.2. Surface determinations

The moulds and yeast count in salamis was evaluated in plates containing the media reported as follows:

Malt extract agar (OXOID), modified with the addition of 0.01% chlorotetracycline (Sigma).

Dichloran rose bengal cloroanphenicol agar (OXOID).

Dichloran glycerol agar base (OXOID).

The tests were carried out on the total casing. This work was made under a Faster Dasit vertical laminar-flow hood and the casing was washed with sterile water containing Tween 80 at 0.1% to obtain a dilution factor of 0.25 (w/w). The plates were incubated at 25°C for 4–7 days , the concentrations relating to individual species are expressed in cfu/cm^2.

The identification of moulds was made according to Pitt (1991); Pitt and Hocking (1985).

2.2. Chemical analyses

2.2.1. Composition

Moisture, salt, proteins and fats according to Association of Official Analytical Chemists (AOAC, 1990)

2.2.2. DL-lactic acid and acetic acid

Ten grams of the sample was added with 30 ml 1 M perchloric acid as deproteinizer in a Sorval Omni-mixer; after homogenization for 2 min at room temperature the slurry is brought to 100 ml with 1 M $HCLO_4$ and centrifugated at 4×10^3 rpm for 5 min at 5°C in 5 ml of filtrate, the pH is adjusted to 8–10 with 2 M KOH, the mixture is then brought to 100 ml with distilled water into a volumetric flask and refrigerated 20 min at 2°C for precipitation of fat. One hundred microlitres of the solution, obtained by filtration through Watman 4 paper, are analysed for DL-lactic acid and acetic acid content using the Boehringer Mannheim enzymatic kit Nos. 1112821 and 148261, respectively.

2.2.3. Free amino acids

The quantitative analyses of free amino acids were performed by HPLC by using two WATERS mod. 510 pumps, a WATERS mod.WISP 717 autosampler and WATERS mod. 474 spectrofluorimeter. The separation was obtained by means of an ionic exchange column (AA Interaction, Lithium form, 6 μm, 12cm × 0.46 cm I.D.) maintained at 39°C.

Two eluents were utilized: Li citrate and Li chloride buffers (pH 2.92 and 10.55). Amino acids were detected by post column derivatization (with two WATERS mod. 501 pumps) first with sodium hypoclorite in a borate buffer (pH = 10) and susbsequently with ortophtalaldehyde (OPA) in a borate buffer (pH = 10). Fluorescence detection: $\lambda_{exc} = 330$ nm, $\lambda_{em} = 440$ nm (Marchelli 1992) and (Dossena, Galaverna, Corradini & Marchelli, 1993)

The enantioselective analyses of D- and L- glutamic acid (Glu), Aspartic acid (Asp), alanine(Ala) and Proline (Pro) were performed by GC–MS by using a Hewlett–Packard mod. 6890 instrument at 70eV equipped either with a CHIRASIL-L-VAL column (Chrompack), or with a chiral tetraamide type selector (L-Phe-3-O-TA) developed in our laboratory. (Marchelli, et al., 1992) and (Gandolfi, Palla, Marchelli, Dossena, Puelli & Salvadori, 1994) For all the analyses, for each sample, the calculated values are the mean of at least three different determinations (3 samples x 3 injections).

2.3. Variations in the principal physicochemical and microbiological parameters as a function of the various drying methods

2.3.1. Recording of the data

In order to be able to effect optimal controls of the operative conditions and consequently to set up correctly the above-mentioned mathematical models, *T* and *RH* measuring probes were placed in various positions inside the cell (*N* 4 *RH* probes and 8 *T* probes) and in the air inlet and take-up channels. The differences measured between the values recorded by the various probes are of fundamental importance for the air circulation model, although this aspect will be addressed in detail in other reports. This report will deal with the *RH* and *T* values obtained from the mean of the measurements of all the probes placed inside the cells.

2.3.2. Ripening tests; differences in the principal thermohygrometric parameters.

In the planning of the tests designed to evaluate the influence of the drying conditions on product characteristics, account was taken of the technologies adopted by

Table 1
Characteristics of salamis

Salami (commercial name)	Country	Duration of ripening (days)	External diametre (mm)	Casing	Grinding size (mm)
Ménage	France	28–32	55–60	Natural: porc	7
Varzi	France	60–65	100	Natural casing obtaining a regular form (tube)	7
Turista	Italy	14-21	55–60	Natural: porc	8
Crespone Milano	Italy	60–70	90	Artificial (collagenic)	3.5
Salchicon Casero	Spain	14–18	50–55	Natural: bovine	5
Salchicon Cular cosido	Spain	28–32	80	Reconstituted collagen. sewed up	5

the individual firms and of the requirements of the project, and in particular:

- the Spanish salamis were always dried at a high relative humidity (*RH*), practically identical in the two series of tests, modifying only the ripening conditions;
- the large Italian and French salamis were dried and ripened at differing RH values, with the salt/water ratio being kept more or less equal;
- the small Italian and French salamis were dried and ripened in similar *RH* conditions and the salt/water ratio was modified.

For both the cells, the drying and ripening of the salamis were effected, as applied by the majority of the companies of the three countries, by varying the RH between a minimum and a maximum value, which differed by up to several tens of percentage points. It was not considered opportune to adopt the constant RH technology, since this is not widely used in the countries taking part in the study. The drying conditions of the two cells were different as to air velocity (higher in cell C01, situated at La Roche sur Foron) and ventilation methods: continuous for cell C02 (situated in Parma) and only in correspondence to the drying periods (passage from the maximum to minimum UR values) for cell C01.

Table 2 reports the means of the minimum, maximum and mean *RH* and *T* values for the various experiments. The ripening cycles were subdivided into two periods (first period or drying, second period or ripening), differing in thermohygrometric conditions; at the end of each one, the products underwent microbiological and physicochemical analyses.

2.4. Examination of the indicators of physicochemical and microbiological parameters; evaluation of the differences as a function of treatment methods

As mentioned in the preceding paragraph, two batches of salami of the same type were matured in the pilot plants, varying some thermohygrometric parameters: essentially, *RH*, air velocity and ventilation methods. Since the evolution during the ripening process of the principal characteristics of the salamis was influenced by a multitude of factors (the quality of the fresh meat, ingredients and additives, treatment of the meat and of the mixture prior to ripening etc.) a comparison between batches of differing products, given the limited number of tests, does not seem possible; thus this report will indicate the results of the individual experiments, avoiding any complex statistical elaboration; the latter will be effected when all the tests are ended, and only significant differences concerning pairs of products of the same type will be recorded.

Table 2
Thermohygrometric conditions of the air in the cells (temperature. *RH* mean, minimum and maximum values) and percentage of weight loss of products ripened in pilot plants

Salami	Days[a]	Cell	Temperature °C	RH mean	RH min	RH max	Weight loss% [b]
Casero1	0-7	C02	14.89	84.09	66.53	90.07	13.15
Casero1	8-14	C02	13.53	75.14	65.90	81.01	30.98
Casero2	0-7	C01	13.81	83.40	64.04	87.84	13.55
Casero2	8-14	C01	13.98	91.88	73.16	92.11	23.5
Cosido1	0-7	C01	15.97	83.56	75.50	88.83	12.86
Cosido1	8-28	C01	14.35	86.57	70.00	94.00	26.74
Cosido2	0-7	C02	15.15	84.57	62.51	88.82	12.5
Cosido2	8-28	C02	12.95	82.14	68.84	83.83	27.3
Crespone1	0-7	C01	16.83	73.21	57.43	87.14	11.7
Crespone1	8-63	C01	13.23	75.60	71.71	82.57	29.57
Crespone2	0-7	C01	17.04	83.90	60.71	95.00	8.46
Crespone2	8-63	C01	12.93	92.22	74.00	98.86	23.56
Ménage1	0-7	C02	16.01	85.92	73.74	95.93	19.7
Ménage1	8-28	C02	13.55	87.97	81.12	92.84	35.74
Ménage2	0-7	C02	16.80	85.73	58.92	91.72	19.65
Ménage2	8-28	C02	13.36	82.47	68.33	85.71	37.85
Turista1	0-7	C01	18.53	72.00	61.00	87.00	15.31
Turista1	8-14	C01	13.86	82.71	76.00	90.29	18.86
Turista2	0-7	C01	19.53	75.00	53.00	86.86	18.07
Turista2	8-14	C01	14.39	83.29	76.57	88.71	23.55
Varzi1	0-7	C02	16.61	87.86	65.67	89.45	12.13
Varzi1	8-63	C02	13.63	81.18	64.47	88.27	35.5
Varzi2	0-7	C02	16.82	78.44	58.46	83.36	13.18
Varzi2	8-63	C02	13.60	83.31	68.70	85.06	34.2

[a] From beginning of drying.
[b] Percentage on weight before drying.

3. Results and discussion

3.1. Surface parameters

3.1.1. Evolution of the surface microbial flora

The physicochemical parameters of the air of the ripening environments directly influence the multiplication of the surface microbial flora, and this is of particular importance for products which are traditionally covered in a patina consisting mainly of moulds and yeasts. The use of selected mould starter cultures, or, more rarely, of yeast ones, is common practice, ensuring an attractive aspect (a mycelium which is white in colour) and the absence of mycotoxins. Among the strains which can be considered, *P. nalgiovense* is very widely used (Leistner, Geisen & Fink-Gremmels, 1989), also in many traditional production methods; it is used regularly as a starter culture by some producers taking part in the project, and is present in the air of environments where the pilot cells have been placed. The outcome of the various experiments in the pilot plants is reported in Table 3 ; significant differences can be observed.

In the evaluation of the results obtained in the individual tests, judgement criteria were adopted which were similar to those normally used in industrial practice; those techniques were considered positive which allowed

Table 3
Surface moulds and yeast counts (log $_{10}$ cfu/cm^2) during the ripening in pilot plants (three sausages for every time)

Salami	Days	*P. nagiovense*			*P. chrysogenum*			*P. gladioli*			*Yeasts*			*Mucor racemosus*		
Casero1	0		1.8			< 0.2			< 0.2			1.8			< 0.2	
Casero1	7	5.4	5.8	5.4	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	6.2	6.6	6.0	1.5	1.9	3.0
Casero1	14	4.8	5.7	6.6	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	6.9	6.4	7.0	< 0.2	< 0.2	< 0.2
Casero2	0	1.8			< 0.2			< 0.2			1.8			< 0.2		
Casero2	7	4.1	4.1	4.0	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	6.1	6.7	6.4	2.8	2.4	3.0
Casero2	14	7.4	7.3	7.5	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	7.4	6.6	6.9	3.4	2.8	3.5
Cosido1	0	4.0			2.1			3.6			3			< 0.2		
Cosido1	7	5.6	5.5	6.0	< 0.2	< 0.2	< 0.2	7.1	7.1	7.2	6.8	6.9	7.0	< 0.2	< 0.2	< 0.2
Cosido1	28	6.4	6.4	6.1	< 0.2	< 0.2	< 0.2	8	8.1	7.9	7.8	7.4	7.4	< 0.2	< 0.2	< 0.2
Cosido2	0	4.0			2.1			3.6			3.0			< 0.2		
Cosido2	7	4	3.9	3.5	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	7.5	7.5	7.4	3.5	< 0.2	3.5
Cosido2	28	6.8	6.7	6	< 0.2	< 0.2	< 0.2	7.4	7.5	7.4	7.3	6.5	7	< 0.2	< 0.2	< 0.2
Crespone1	0	0.7			< 0.2			1.9			1.9			< 0.2		
Crespone1	7	2	2.4	2.3	2	2.8	2.3	4.3	3.7	3.7	4.7	4.2	4.3	< 0.2	< 0.2	< 0.2
Crespone1	63	3.8	4.8	4.1	4.8	5.4	4.1	5.3	6.6	5.5	5.5	5.5	5.5	< 0.2	< 0.2	< 0.2
Crespone2	0	2.1			< 0.2			3.9			4.1			< 0.2		
Crespone2	7	5.6	5.7	5.4	6.3	5.5	6	7.4	7.2	7.1	5.7	5.4	5.6	< 0.2	< 0.2	< 0.2
Crespone2	63	[a]	[a]	[a]	5.7	5.7	5.7	7.8	6.5	7.3	7.2	7.1	6.9	< 0.2	< 0.2	< 0.2
Ménage1	0	0.2			< 0.2			< 0.2			3.2			< 0.2		
Ménage1	7	4.9	5.9	4.8	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	7	7.5	7.4	2.2	2.7	2.4
Ménage1	28	6.6	7.1	7.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	7.4	7.4	7.3	< 0.2	< 0.2	< 0.2
Ménage2	0	2.5			1.0			3.1			3.3			< 0.2		
Ménage2	7	5	5.9	5.5	3	< 0.2	3	< 0.2	< 0.2	< 0.2	6.4	6.3	6.1	< 0.2	< 0.2	< 0.2
Ménage2	28	7.1	7.1	7.1	< 0.2	< 0.2	< 0.2	5.9	6.5	6.7	6.3	7.1	6.9	< 0.2	< 0.2	< 0.2
Turista1	0	3.9			< 0.2			< 0.2			3.8			< 0.2		
Turista1	7	3.7	4.6	3.7	< 0.2	< 0.2	< 0.2	3.6	3.8	2.8	5.6	5.6	5.1	< 0.2	< 0.2	< 0.2
Turista1	14	6.1	6.2	6	< 0.2	< 0.2	< 0.2	5.3	5.2	5.2	5.5	5.8	5.9	< 0.2	< 0.2	< 0.2
Turista2	0	3.2			1			< 0.2			2.9			< 0.2		
Turista2	7	4.5	4.4	4	4.9	4.5	3.8	5.4	4.9	4.6	4.6	4.5	4.4	< 0.2	< 0.2	< 0.2
Turista2	14	5.8	5.8	5.6	6.4	6.7	5.7	7	6.7	6.2	5.6	5.5	5.3	< 0.2	< 0.2	< 0.2
Varzi1	0	0.2			< 0.2			< 0.2			< 0.2			< 0.2		
Varzi1	7	5.7	6	5	4.6	4.9	4.6	< 0.2	< 0.2	< 0.2	6.9	6.7	6.8	< 0.2	< 0.2	< 0.2
Varzi1	63	6.4	6.5	5.8	6.4	5.9	6.1	7.5	7.9	7.1	7.2	7.4	7.4	< 0.2	< 0.2	< 0.2
Varzi2	0	1.6			< 0.2			< 0.2			< 1.2			< 0.2		
Varzi2	7	4.7	4.8	<	<	<	< 0.2	< 0.2	< 0.2	< 0.2	6.3	5.8	5.9	< 0.2	< 0.2	< 0.2
Varzi2	63	7.1	7.4	7.3	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	6.3	6.5	6.4	< 0.2	< 0.2	< 0.2

[a] Not detectable.

for the growth of moulds considered characteristic (essentially *P. nalgiovense*) and which inhibited the multiplication of others, with particular attention to those which are potentially toxinogenic and/or with a mycelium of an undesirable colour.

As concerns toxinogenic moulds, in no case was the presence revealed of types definitely producing mycotoxins. Of the moulds which were different from *P. nalgiovense*, the various types of *P. Gladioli* are normally considered acceptable, being non-toxinogenic and with a mycelium of a grey colour; *P. chrysogenum* was not considered acceptable, since some strains can provide a mycelium of a green colour; again on account of aesthetic problems, the various strains of *Mucor* were also excluded.

In all the salamis, yeasts were always found in large quantities, since these micro-organisms are not so dangerous as moulds and do not normally significantly influence the external aspect. However, the high quantities of yeasts in all the products and some of their characteristics (low volatility, attractive aspect, etc.) warrant a more thorough specific investigation.

An initial examination of the data shows the existence of a certain difference as a function of ripening times; the matured salamis of greater size and longer ripening times normally present an initial multiplication characterised essentially by positive moulds (essentially *P. nalgiovense*), while towards the end of the ripening period there is the prevalence of other strains (*P. gladioli, P. chrysogenum)*.

The multiplication of *P. nalgiovense* in the first days of ripening is favoured by the relatively high *RH* values.

Of the two types of plant management, although the data are not sufficiently numerous, it would seem that greater air velocities accompanied by interruptions in

ventilation lead to better results, especially for the products of smaller size. These data underline several interesting aspects:

- the poor competitiveness of *P. nalgiovense* compared with other contaminants requires a low rate of environmental contamination;
- better results are obtained by using higher mean RH values, albeit within the framework of the values adopted for these tests; in fact, higher values could favour the multiplication of other strains which are also potentially toxinogenic;
- better surface flora is obtained with higher air velocities and by stopping the ventilation according to regular cycles.

3.1.2. Variation in the quantity of water in the internal and external fractions during the ripening period

Although the parameters considered previously depend on numerous factors, mainly linked to the composition and size of the salamis, the variation in water quantity in the various fractions should be more directly connected to the thermohygrometric conditions of the air in the cells (temperature, *RH* and velocity).

For all the products, two concentric fractions were examined, the more internal one with a radius practically equal to the dimension of the external circular crown; this subdivision was necessary because of the considerable difference between the velocity of evaporation and diffusion of the water, which can give rise to significant differences in the $NaCl/H_2O$ ratio of the

Table 4
Variation of percentage of moisture and salt in two concentric fractions by ripening of salamis in pilots plants

Salami	Days	Moisture int[a]		Salt inta		$(NaCl/H_2O)$ 100 int[a]	Moisture ext[a]		Salt ext[a]		$(NaCl/H_2O)$* 100 ext[a]
		Mean	S.D.	Mean	S.D.	Mean	Mean	S.D.	Mean	S.D.	Mean
Casero1	0	53.98	0	2.22	0	4.11	53.98	0	2.22	0	4.11
Casero1	7	44.55	0.81	2.79	0.14	6.26	39.55	1.86	3.11	0.06	7.86
Casero1	14	34.83	0.64	3.03	0.34	8.70	30.28	0.74	3.16	0.16	10.44
Casero2	0	53.98	0.00	2.22	0.00	4.11	53.98	0.00	2.22	0.00	4.11
Casero2	7	50.62	0.96	2.60	0.17	5.14	42.01	0.84	2.29	0.08	5.45
Casero2	14	38.73	0.56	2.65	0.04	6.84	28.04	0.23	2.15	0.06	7.67
Cosido1	0	57.20	0.00	2.51	0.01	4.39	57.20	0.00	2.52	0.00	4.41
Cosido1	7	53.40	1.40	2.48	0.18	4.64	45.93	2.09	2.24	0.17	4.88
Cosido1	28	44.99	1.24	2.82	0.05	6.27	35.98	1.65	1.83	0.19	5.09
Cosido2	0	57.20	0.00	2.51	0.01	4.39	57.20	0.00	2.52	0.00	4.41
Cosido2	7	58.36	0.76	2.52	0.12	4.32	54.03	0.23	2.52	0.09	4.66
Cosido2	28	45.46	2.42	3.11	0.42	6.84	35.50	1.15	2.22	0.55	6.25
Crespone1	0	55.94	0.68	2.77	0.07	4.95	55.60	0.33	2.77	0.07	4.98
Crespone1	14	54.68	0.86	3.51	0.09	6.42	46.13	0.50	3.45	0.08	7.48
Crespone1	63	46.91	0.12	5.00	0.12	10.66	34.78	0.15	4.00	0.06	11.50
Crespone2	0	54.42	0.47	2.99	0.03	5.49	54.42	0.47	2.99	0.03	5.49
Crespone2	14	54.13	0.39	3.27	0.18	6.04	49.17	0.52	3.05	0.12	6.20
Crespone2	63	50.83	0.31	4.45	0.09	8.75	41.15	0.54	3.71	0.17	9.02
Ménage1	0	58.67	0.01	3.24	0.48	5.52	58.67	0.01	3.24	0.48	5.52
Ménage1	7	55.45	0.77	4.05	0.08	7.30	47.05	1.36	3.87	0.08	8.23
Ménage1	28	44.25	0.87	5.15	0.21	11.64	34.46	1.69	4.07	0.24	11.81
Ménage2	0	59.20	0.68	2.71	0.02	4.58	59.20	0.68	2.71	0.02	4.58
Menage2	7	55.20	0.76	3.62	0.01	6.56	46.01	0.36	2.98	0.04	6.48
Menage2	28	42.41	0.35	5.22	0.07	12.31	32.32	0.72	3.56	0.05	11.01
Turista1	0	53.08	1.04	3.02	0.02	5.69	53.08	1.04	3.02	0.02	5.69
Turista1	7	51.26	0.22	3.57	0.37	6.96	42.38	0.72	3.25	0.18	7.67
Turista1	14	49.15	1.52	4.42	0.20	8.99	39.50	0.98	3.92	0.17	9.92
Turista2	0	57.02	0.03	2.75	0.24	4.82	57.02	0.03	2.75	0.24	4.82
Turista2	7	53.61	1.24	3.71	0.11	6.92	45.58	0.08	3.43	0.08	7.53
Turista2	14	51.23	1.70	4.31	0.25	8.41	41.35	0.83	3.53	0.12	8.54
Varzi1	0	59.21	0.03	2.74	0.09	4.63	59.21	0.03	2.74	0.09	4.63
Varzi1	7	58.66	0.57	3.32	0.05	5.66	50.23	0.12	3.19	0.04	6.35
Varzi1	63	42.24	0.71	5.29	0.17	12.52	30.19	0.61	3.82	0.09	12.65
Varzi2	0	57.61	0.13	2.56	0.03	4.44	57.61	0.13	2.56	0.03	4.44
Varzi2	7	55.00	1.06	3.33	0.05	6.05	47.50	0.68	3.19	0.02	6.72
Varzi2	63	43.76	0.56	5.09	0.08	11.63	33.90	0.70	3.82	0.05	11.27

[a] int = Internal fraction; ext = External circular crown.

Table 5
Variation of pH in two concentric fractions of sausages ripened in pilot plants

Salami	Days	pHint		pHext	
		Mean	S.D.	Mean	S.D.
Casero 1	0	6.14	0	6.14	0
Casero1	7	4.90	0.03	5.13	0.07
Casero1	14	5.25	0.07	5.50	0.11
Casero2	0	6.14	0.00	6.14	0.00
Casero2	7	4.65	0.12	4.95	0.03
Casero2	14	4.90	0.03	5.33	0.08
Cosido1	0	6.18	0.00	6.18	0.00
Cosido1	7	4.91	0.05	5.01	1.53
Cosido1	28	4.70	0.07	4.91	1.51
Cosido2	0	6.18	0.00	6.18	0.00
Cosido2	7	4.73	0.04	4.79	0.01
Cosido2	28	4.66	0.08	4.70	0.02
Crespone1	0	6.05	0.00	6.05	0.00
Crespone1	7	5.19	0.01	5.31	0.02
Crespone1	14	4.95	0.03	5.17	0.06
Crespone1	63	5.11	0.06	5.62	0.05
Crespone2	0	5.95	0.00	5.95	0.00
Crespone2	7	5.03	0.03	5.16	0.04
Crespone2	14	5.22	0.04	5.46	0.06
Crespone2	63	5.30	0.04	5.74	0.08
Ménage1	0	5.84	0.01	5.84	0.01
Ménage1	7	5.11	0.01	5.19	0.05
Ménage1	28	5.07	0.01	5.24	0.04
Ménage2	0	6.00	0.06	6.00	0.06
Ménage2	7	5.01	0.02	5.13	0.01
Ménage2	28	5.37	0.03	5.59	0.03
Turista1	0	5.71	0.00	5.71	0.00
Turista1	7	5.34	0.06	5.48	0.10
Turista1	14	5.31	0.02	5.47	0.03
Turista2	0	6.03	0.00	6.03	0.00
Turista2	7	5.18	0.01	5.33	0.05
Turista2	14	5.25	0.02	5.35	0.06
Varzi1	0	5.93	0.01	5.93	0.01
Varzi1	7	4.82	0.02	5.06	0.03
Varzi1	63	4.85	0.01	4.99	0.07
Varzi2	0	5.87	0.00	5.87	0.00
Varzi2	7	4.74	0.01	4.91	0.02
Varzi2	63	4.79	0.05	5.06	0.09

various fractions and thus in microbial multiplication and in the evolution of many connected chemical and physico-chemical parameters (e.g. pH, proteic hydrolysis etc.). Comparing the variation in the quantity of water and in the salt/water ratio in the various fractions at the end of the drying and ripening stages, the following observations can be made:

- drying is much more marked in the external fractions and considerably lower in the internal ones;
- the quantity of water is always lower in the external fractions;
- the quantity of salt is always lower in the external fractions;
- the salt/water ratio is always greater in the external fractions at the end of the drying stage and tends to be equal in the various fractions at the end of the maturing stage.

These observations are not completely in agreement with the laws of diffusion, according to which there should be uniformity of the salt/water ratio in all the fractions of the salami; a more detailed examination of the data may give rise to more interesting conclusions:

(1) Large salamis:
- lower RH values cause greater water loss and a greater increase in the salt/water ratio in the external fractions and, consequently, also a higher salt/water ratio in the internal fractions;
- the phenomena are more marked with the adoption of ventilation in continuum (crespone: cell 01); the quantity of water in the internal fractions of the bigger salamis undergoes considerably smaller variations in the first ripening period, where the transformations are greater (Table 4).

(2) Small salamis:
- a greater quantity of water and/or a smaller quantity of salt in the fresh product will give rise to a greater loss of water in the external fractions at the end of the drying stage and a greater increase in the salt/water ratio in all the fractions. In any case, the salt/water ratio is always greater in samples in which this parameter was also higher in the fresh mixture.

3.1.3. Variation of pH in internal and external fractions

All the salamis presented a significant amount of acidification during the first period of ripening, as confirmed by the onset of fermentation processes. Acidification was higher in the salamis of bigger size and, referring to the various samples, higher in the Spanish and lower in the Italian ones. (Table 5)

3.1.4. Internal microbial flora

All the salamis in the experiment were inoculated with starter cultures consisting of micrococcaceae (all the products) and lactobacilli (French and Italian products) and pediococci (Spanish salamis); the analyses were designed to verify the multiplication of the characteristic micro-organisms (micrococcaceae and lattobacillaceae), the inactivation of the normal contaminating agents (Gram- etc.)

3.1.5. Short chain organic acids

In all the salamis examined (Italian and French products), a significant increase in the D-lactic and acetic acid content was to be observed, whereas the increase in L-lactic acid was lower (Table 6).

3.1.6. Amino acid production

The quantity of free amino acids increases during the ripening; the increase is proportionally greater in the drying period and less marked during ripening. Although the two tests on the bigger-size Spanish salamis (salchicon cular cosido) show no significant differences concerning the quantity of free amino acids at the end of the ripening period, it was a different picture for the Italian and French products; in particular, batch crespone 1 presented much higher quantities than batch crespone 2, similarly to the situation for turista 2 and menage 1. As regards the absolute quantities, the greater the ripening time, the greater the increase in the quantity of free amino acids (Table 7).

Table 6
Variation of D-lactic, L-lactic and acetic acid values by ripening in French and Italian salamis (pilot plant)

		D-Lactic acid (%)		L-Lactic acid (%)		Acetic acid (%)	
Salami	Days	Mean	S.D.	Mean	S.D.	Mean	S.D.
Crespone1	0	0.004	0.002	0.491	0.016	0.008	0.001
Crespone1	14	0.626	0.042	0.624	0.031	0.033	0.025
Crespone1	63	0.829	0.023	0.769	0.015	0.071	0.005
Crespone2	0	0.007	0.002	0.556	0.009	0.007	0.001
Crespone2	14	0.552	0.012	0.550	0.008	0.042	0.002
Crespone2	63	0.592	0.055	0.549	0.055	0.089	0.004
Ménage1	0	0.168	0.018	0.291	0.008	0.020	0.001
Ménage1	7	0.384	0.022	0.470	0.021	0.054	0.007
Ménage1	28	0.515	0.017	0.562	0.024	0.039	0.004
Ménage2	0	0.187	0.002	0.229	0.007	0.022	0.002
Ménage2	7	0.364	0.004	0.438	0.004	0.070	0.001
Ménage2	28	0.497	0.007	0.552	0.004	0.040	0.004
Turista1	0	0.008	0.001	0.618	0.006	0.014	0.007
Turista1	7	0.548	0.027	0.511	0.012	0.050	0.005
Turista1	14	0.605	0.026	0.628	0.016	0.061	0.011
Turista2	0	0.051	0.009	0.546	0.014	0.007	0.004
Turista2	7	0.611	0.007	0.657	0.010	0.054	0.002
Turista2	14	0.708	0.031	0.656	0.020	0.080	0.004
Varzi1	0	0.170	0.021	0.271	0.013	0.004	0.002
Varzi1	7	0.366	0.007	0.446	0.008	0.056	0.005
Varzi1	63	0.669	0.005	0.359	0.003	0.059	0.001
Varzi2	0	0.170	0.021	0.207	0.153	0.022	0.003
Varzi2	7	0.369	0.013	0.432	0.028	0.061	0.002
Varzi2	63	0.858	0.004	0.654	0.027	0.106	0.010

As previously shown by researches carried out in the laboratories of the Department of Organic and Industrial Chemistry of the University of Parma, D-Ala, D-Asp and D-Glu can be considered as markers of fermentation and ripening processes. (Gandolfi, et al., 1994), (Palla, Marchelli, Dossena, & Casnati, 1989), (Marchelli, Dossena, & Palla, 1996) We decided, therefore, to monitor the amounts and the percentages (D/D + L) of these enantiomers in order to verify if they can be considered as markers of fermentation also in salamis. The data obtained from these analyses confirmed that the ripening process in salamis can be correlated to the enantiomeric ratio of D- and L- amino acids.

The statistical analyses of these data could provide a general and sound methodology to asses the ripening state of salamis. Since the Ds are pertaining to the metabolism of the micro-organisms present in the salamis, while the Ls also derive from the enzymatic demolition of the proteins, this opens up interesting prospects in the research regarding this subject.

Table 7
Variation of the amount (mg/100 g of dry matter) of free D-alanine. D-glutamic acid and D-aspartic acid in salamis by ripening (pilot plants)

Salami	Days		D-AlaL	D-Asp	D-GluLF	Total D-amino acids	Total amino acids
Casero2	14	Mean	8.47	1.77	7.43	17.67	414.20
		S.D.	0.50	0.15	0.70		
Cosido1	28	Mean	16.87	5.40	12.03	34.30	614.10
		S.D.	1.50	0.40	1.21		
Cosido2	28	Mean	17.50	3.97	14.47	35.93	613.37
		S.D.	0.35	0.25	0.49		
Crespone1	0	Mean	1.47	0.00	0.40	1.87	310.5
		S.D.	0.25	0.00	0.10		
Crespone1	14	Mean	10.00	2.07	5.67	17.73	749.67
		S.D.	1.15	0.23	0.87		
Crespone1	63	Mean	13.20	4.40	12.80	30.40	1141.87
		S.D.	0.40	0.40	1.25		
Crespone2	0	Mean	0.00	0.00	0.23	0.23	302.44
		S.D.	0.00	0.00	0.06		
Crespone2	14	Mean	11.83	1.87	4.60	18.30	654.36
		S.D.	0.15	0.25	0.17		
Crespone2	63	Mean	17.60	3.63	11.87	33.10	846.20
		S.D.	1.47	0.75	1.59		
Menage1	28	Mean	13.43	3.80	5.67	22.90	740.80
		S.D.	0.68	0.10	0.21		
Menage2	28	Mean	10.33	2.70	3.90	16.93	476.23
		S.D.	0.93	0.26	0.36		
Turista1	0	Mean	0.00	0.00	0.37	0.37	274.84
		S.D.	0.00	0.00	0.06		
Turista1	7	Mean	5.87	0.70	1.43	8.00	383.09
		S.D.	0.47	0.10	0.12		
Turista1	14	Mean	7.27	1.27	1.97	10.50	402.53
		S.D.	0.50	0.15	0.12		
Turista2	0	Mean	0.00	0.00	0.27	0.27	260.49
		S.D.	0.00	0.00	0.06		
Turista2	7	Mean	11.07	1.57	3.87	16.50	497.93
		S.D.	0.29	0.06	0.21		
Turista2	14	Mean	13.03	1.90	5.57	20.50	565.56
		S.D.	0.93	0.10	0.76		
Varzi1	63	Mean	20.97	5.00	6.97	32.93	991.27
		S.D.	1.50	0.36	0.42		
Varzi2	63	Mean	20.60	5.00	4.77	30.37	1104.73
		S.D.	4.22	0.20	2.02		

3.1.7. Influence of the salt/water ratio on the formation of free amino acids and on D-amino acids

Comparing paired samples of salamis of the same type, it can be observed that the differences in the quantities of the differing D-amino acids are slight, seemingly of significance only in some cases (Turista and Menage); for these samples, it can be noted that those with higher quantities of D-amino acids also have a lower salt/water ratio.

The same observations can also be made for acetic acid, which has a direct influence on certain sensorial properties, such as the piquant flavour of matured salami.

4. Conclusions

The DRIP project set out to improve the drying and ripening techniques of salamis; the subject has been examined from several different angles.

The experiments concerning the influence of drying conditions on the multiplication of the surface microbial

flora resulted as being of particular importance. As regards *RH*, the best results were obtained for relatively high values near to the optimal conditions of the starter cultures used (*P. nalgiovense*). The experimental data would seem to indicate that the alternating of ventilation cycles at high air velocities with cycles in which the product is allowed to rest leads to better growth of the starter moulds than in trials where there is a continuous circulation of air.

Independently of the thermohygrometric conditions, these micro-organisms do not always become the dominant population, especially if the periods of ripening are long. In the presence of a limited number of other contaminants in the fresh product and in correct thermohygrometric conditions, there is initially a good multiplication of the starter cultures, whereas towards the end of the ripening process the dominant population more often than not consists of other moulds Since drying methods, and in particular the mean *RH* value and ventilation methods, seem to influence the variation in the salt/water ratio and, consequently, in the others parameters (free D-amino acid, short chain organic acids) termoygrometric conditions should be chosen with care so as to obtain products with the best qualitative characteristics; to obtain these results, the use of mathematical models of the heat and mass transfer, which will be reported in subsequent works, will be of assistance in defining the optimal technologies.

References

Association of Official Analytical Chemists. *Official methods of analysis* (15th ed.). Arlington, VA: Association of Official Analytical chemists.

Campbell-Platt, G., & Cook, P. E. (1995). *Fermented meats*. Glasgow: Blackie Academic and Professional.

Dossena, A., Galaverna, G., Corradini, R., & Marchelli, R. (1993). Two dimensional high-performance liquid chromatographic system for the determination of enantiomeric excess in complex amino acid mixtures. *Journal of Chromatography, 653*, 229–234.

Gandolfi, I., Palla, G., Marchelli, R., Dossena, A., Puelli, S., & Salvadori, C. (1994). D-alanine in fruit juices : a molecular marker of bacterial activity, heat treatment and shelf-life. *Journal of Food Science, 59*, 152–154.

Leistner, L., Geisen, R., & Fink-Gremmels, J. (1989). In: S. Natori, K. Hashimoto & Y. Ueno. *Micotoxins and phycotoxins '88'* (p.145). Amsterdam: Elsevier Science Pubbliser B.V.

Marchelli, R., Dossena, A., Palla, G., Audhuy-Peaudecerf, M., Lefeuvre, S., Canevali, P., & Freddi, M. (1992). D-amino acids in reconstituted infant formula : a comparison between conventional and microwave heating. *Journal of Science and Food Agriculture, 59*, 217–226.

Marchelli, R., Dossena, A., & Palla, G. (1996). The potential of enantioselective analysis as a quality control tool. *Trends in Food Science and Technology, 7*, 113–119.

Palla, G., Marchelli, R., Dossena, A., & Casnati, G. (1989). Occurrence of D-amino acids in food: detection by capillary GC adn by reversed-phase HPLC with L-phenylalaninamides as chiral selectors. *Journal of Chromatography, 475*, 45–53.

Pitt, J. I., & Hocking, A. D. (1985). *Fungi and food spoilage*. Sidney, New York, London: Academic Press.

Pitt (1991). *A laboratory guide to common Penicillium species*. Australia: CSIRO Food Research Laboratoty, North Ryde.