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CHANGES PRODUCED DURING CURING OF DRY SAUSAGES

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## CHANGES PRODUCED DURING CURING OF DRY SAUSAGE,

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Since the first work of Cesari (1919) on curing of dry sausages, many reports have been published covering both the role played by microorganisms in fermentation processes and biochemical changes during curing inducing specific flavor and aroma.

Regarding the first point, the research work by Niinivaara (1957), Niinivaara and Pohja (1957 a and b), Pohja and Gylleberg (1962), Ingram and coworkers (1957), etc., have cleared up many circumstances on the occurrence of several bacterial genera and its role during maturation.

In order to add new data or experiences in this field, we undertook experiments with the Spanish dry sausages named "chorizo", because real knowledge of the maturation processes and factors related are lacking. This type of sausage represents the highest amounts of cured meats in our country, including the different regional varieties. Differences among these varieties refer to flavor and presentation and except industrial production in some big factories, this product is processed for familiar consumption and hence the variation in character of the final product.

Generally speaking, the production is located in the mountains region, due to climate conditions needed for curing.

### MATERIALS AND METHODS

Dry sausage "chorizo". The specimens were prepared under commercial conditions in a factory, that normally processes this product, named "Revilla", that kindly supply it.

The way in which "chorizo" was obtained throughout the following operations :

After refrigeration carcasses were divided and separation was made according to the quality of the different parts.

The proper region of pig and beef carcasses were trimmed together to get pieces ranging from 0.5 to 1.5 cm.

After trimming salt, nitrates and different seasoning were added (paprika, garlic powder, mainly), sugar and in some cases, polyphosphates too.

At this moment, normally, the meat emulsion is kept overnight at 4 to 6° C, and natural casing of 30-34  $\phi$  cm. were used afterwards.

Curing process is preceded by stuffing for periods ranging between 2 to 4 days at 22° C and 85 % RH. Proper curing proceeds at 16° C and 75 % RH, afterwards.

During stuffing some oak smoke is generated in the room.

We have studied three different lots which differs mainly as follows :

Lot 1. - We have used the product as is processed normally in the factory. In this case we were not able to control temperature and humidity during curing because in spite of the big production of this factory, they employ natural air, and very big fluctuations take place daily.

Lot 2. - This lot differs from the first one, because no time was elapsed in the refrigerator, between emulsion obtainment and casing.

This lot was subdivided into two lots, one of which was cured under normal conditions in the factory and the other transported to our Institute to be cured at 14° C  $\pm 0.5$  and 70 per cent relative humidity  $\pm 2$  per cent, after stuffing for a period of 60 hours at 22° C and 80-85 per cent relative humidity.

Both lots were divided again into four ones, as follows :

- 1 - Control
- 2 - seeded with microorganism named "L" !?
- 3 - " " " " "1-6"
- 4 - " " both microorganisms.

The amount of the inoculum was  $8 \times 10^5$  microorganisms per gram of meat emulsion.



Microbiological analysis. -

They were performed as follows :

Samples of the middle zone and the zone nearest to the surface of sausage were taken off with the help of pincers and scissors, aseptically.

Samples are introduced into a modified blender containing 100 c.c. sterile saline solution. The beaker of this blender was made of aluminium and tightly taped with aluminium foil.

After mixing, dilutions and seeding were normally carried out.

The medium employed to pour Petri dishes was constituted as follows :

Agar n<sup>o</sup> 3\* 10 g., peptone\* 5 g., meat extract\* 3 g., glucose 10 g., yeast extract\* 3 g., sodium chloride 30 g. and water up to 1000 c.c.

(\* All these products were "Oxoid")

The incubation was extended during three days at 25° C.

After growing, the number of colonies was taken as viable microorganisms.

Equilibrium relative humidity.-

We follow substantially the method of Kvaale and Dalhoff (1953).

Total humidity.-

Weights were recorded before and after drying for three hours at 100-105° C.

pH.-

A pHmeter 22 "Radiometer" was employed, and dilution of the samples was made with sodium iodoacetate 0.005 M.

Chloride content.-

We employed the Volhard's method as described by A.O.A.C.'s (1960).

RESULTSMicrobiological analyses.-

As can be seen from tables 1 and 2 very high differences are evident between lots.

In the first lot - sausage processed as is normal. in the factory - some type of lag phase could be found during stuffing. After this period a level with log. value of 9.0 is reached and no big variations occurred during curing as referred to the total number of microorganisms.

High constancy could be seen in the difference between the number of viable microorganisms in the zone of the middle and in the zone nearest to the surface. As is shown by values, higher counts have been obtained in the zone nearest to the surface. An effect of oxygen level must be taken into account.

As the time of curing increased, a change took place as referred to the bacterial genera. At the beginning of the curing process we find out among others the following genera : Micrococcus, Pseudomonas, Proteus, Achromobacter, Brevibacterium, Arthrobacter, etc., but at the end of the curing period only two types of bacteria are evident.

Their characteristics are not quite the same as of any of the bacteria described in the "Bergey's Manual (1957)", because of their cultural and biochemical properties. The following observations were made referring to the two types of bacteria :

"L" - Rod shaped, in pairs of changing to coccoid after 24 hours, at 25° C, 0.5 x 2.0 microns, gram-negative. Circular colonies up to 2 mm. on agar, no mobile. On agar slants, small circular colonies, with smooth and glistening surface, sediment in broth, by puncture in agar grows in surface and depth. Aerobic to microaerophilic, does not produce SH<sub>2</sub>, indol neither does change litmus milk but slightly, or reduce nitrates, methyl red and Voges Proskauer tests are negative, acid and gas from glucose, galactase, sucrose, lactose, levulose, mannose, no changes in starch, good growing in broth with added 7 per cent sodium chloride. Optimum temperature 25° C.

The morphological characteristics resemble the Arthrobacter genera. It seems that is the only one with ability to hydrolyzed sugars of all the isolated microorganisms.

"1-6" - Spherical cells, about 1 micron in diameter or less, singly, in pairs or tetrads, gram-positive, small white glistening colonies. Aerobic to microaerophilic.

lic, does not produced  $\text{SH}_2$ , indol, no changes in litmus milk, Methyl Red and Voges Proskauer test are positive, no fermentation action on sugars only slight on fructose, starch unchanged, nitrites and nitrogen produced from nitrates sediment in broth with added 7 per cent sodium chloride. Optimum temperature 20-22° C.

In any ways resembles micrococcus halodenitrificans, and we suppose that is a variant.

Is the only one isolated from sausages with a definite action on nitrates.

These two microorganisms were liophilized and used as starter in the second experiment.

In the second group of lots in which we avoid resting in cold room overnight we are not able to see any lag phase as well in product, cured in factory as in the one processed in our laboratory. The initial numbers of microorganisms were higher than in the first lot.

As the starter was added in amount of  $8 \times 10^5$ , on normally contaminated emulsion, no evidence was found of differences between lots, as referred to the total number of microorganisms, but other differences related to curing process became evident.

In the lots cured under control conditions in our laboratory a shorter period was found to reach the "cured level".

No differences were evident as referred to the genera present.

#### Equilibrium relative humidity.-

The values referred to equilibrium relative humidity are summarized in tables 3 and 4.

Values belonging to lot 1 and 2 cured in factory showed, high differences between the equilibrium relative humidity of the middle zone and that of the zone nearest to the surface. Higher dehydration took place on the surface without normal water transport from the inside. In those sausages cured in our laboratory closer values were obtained for the two zones, and lower values were reached more quickly than in the other lots.



The values at the end of the curing process were around 94 per cent.

#### Total humidity.-

Here we have not the same picture as before with reference to total humidity, as it is shown in tables 5 and 6.

Regarding this point, in all lots an increase in curing time determines higher differences between the zones above considered. Generally speaking, a difference of 10 per cent could be found at the end of the curing process as determined by this practice. To such an extent that we have seen that this difference indicates the end of the curing process.

It can be noted that when curing time goes beyond the desirable level, some differences could be found among total humidities, that are higher in the three lots with added microorganisms.

For sausages of lot 1, resting overnight before casing a quicker water loss than in the case of not resting sausages was apparent when the desirable cured level is reached.

It seems from the tables that some erratic results were obtained. This was due because at the beginning we took the total sausage for humidity estimation. Thereafter we took the two zones above mentioned.

With reference to the weight losses as it is shown in tables 7 and 8, higher amounts are reached for the same curing period, in sausages obtained from meat emulsion resting overnight.

#### pH.-

In tables 9 and 10, we see how pH values have changed during curing process.

In all lots a decrease in pH values has occurred up to a level of 4.7 - 4.9, approximately.

As to the establishment of constant values zone of pH, some differences among lots may be observed.

It was necessary ten days for lot 1 to reach the equilibrium zone. Nevertheless in the other lots - those not resting overnight - the equilibrium zone was

reached in a shorter time, both sausages cured in the factory and those cured in the laboratory .

Closer values were obtained for sausages cured under controlled conditions,

No great differences between lots added and not with microorganisms have been observed.

Sodium chloride content.-

As it may be assumed weight losses during curing process, resulted in higher sodium chloride concentrations, as is shown in table 11.

Due to the same cause, differences in sodium chloride content, between outer and inner layers were produced.

CONCLUSIONS.

The most important fact in curing is the transformation of raw products as a cause of maturation.

In this phenomenon the first role is played by microorganisms present during process and hence the factors affecting its development. In our case among others the redox conditions ceated by paprika must be played a significant role.

From our study it may concluded, that there are two types of microorganisms responsible of maturation, germ " L " that seems to belong to the Arthrobacter genus, and germ " 1-6 ", probably similar to Micrococcus halodenitrificans.

It seems that during curing process, the conditions produced enhance the growth of these two types of bacteria, and at the end of the process these two only are present in the product.

The overnight resting seems to delay the curing process.

Aerobic conditions improve microorganisms growth, as may be seen in table 1, where normal cultures present higher accounts than microaerophilic ones. This a assetr was confirmed too, by the higher accounts notated in zones near surface, in spite of the poorer conditions produced by higher taes of dessication.



T A B L E 1  
L O T. 1

Microorganisms during curing  
log. number

Curing time	Normal		Microaerophilic	
	c <sup>(1)</sup>	s <sup>(2)</sup>	c <sup>(1)</sup>	s <sup>(2)</sup>
0	7.47			
1	7.79			
2	7.20			
6	8.53	8.76	8.49	8.68
10	9.08	9.44	8.70	8.87
14	8.68	9.00	8.73	8.94
20	8.81	9.08	8.66	8.96
24	8.74	8.90	8.65	9.04

(1) middel zone

(2) zone nearest surface

T A B L E 2  
L O T 2

Microorganisms during curing  
log. n°

Sub-lot n°	Curing time								
	1	4	6	7	9	11	12	16	19
1 <sup>c</sup>					9.20		8.53	9.23	8.65
s			8.97		9.95		8.63	9.55	8.39
2 <sup>c</sup>	11.14	9.32		9.20		8.84			
s				9.95		8.82			
3 <sup>c</sup>					8.79		8.50	9.23	8.34
s			9.30		9.56		8.44	10.74	8.82
4 <sup>c</sup>	9.80	8.95		8.70		9.71			
s				11.00		8.80			
5 <sup>c</sup>					8.54		8.08	10.00	8.39
s			9.80		9.04		8.76	10.17	8.41
6 <sup>c</sup>	9.70	9.59		9.00		8.79			
s				9.34		8.76			
7 <sup>c</sup>			9.41		8.84		8.78	8.56	7.87
s					9.04		8.81	10.95	7.90
8 <sup>c</sup>	9.32	9.65		9.54		8.70			
s				9.65		8.74			

\* cured in factory \*\* in laboratory

TABLE 3  
 LOT 1  
 Equilibrium relative  
 humidity %

Curing time	Percentage	
	c	s
2	98.5	
6	99.0	95.5
10	95.5	95.0
14	94.5	91.5
20	90.5	87.0
24	90.5	87.0

TABLE 4  
 LOT 2  
 Equilibrium relative  
 humidity %

Sub- lot n°	Curing time									
	2	4	6	7	9	11	12	14	15	19
1c			97.5		98.5		95.5		91.5	90.5
s					97.5		95.5		91.5	90.5
2c	99.0	97.5		95.5		94.5		91.5		
s				95.5		92.5		90.5		
3c			97.5		98.5		95.5		94.5	91.5
s					97.5		94.5		90.5	90.5
4c	99.0	97.5		95.5		95.5		91.5		
s				95.5		94.5		91.5		
5c			97.5		98.5		95.0		91.5	91.5
s					97.5		91.5		91.5	87.0
6c	99.0	97.5		95.5		94.5		91.5		
s				95.5		94.5		91.5		
7c			97.5		98.5		95.5		91.5	91.5
s					97.5		92.5		91.5	87.0
8c	99.0	97.5		95.5		95.5		91.5		
s				95.5		94.5		90.5		

TABLE 5  
LOT 1  
Total humidity

Curing time	Percentage	
	c	s
2		54.4
6	54.9	50.0
10	53.9	43.1
14	42.9	28.8
20	37.9	29.0
24	33.4	27.0

TABLE 6  
LOT 2  
Total humidity %

Sub- lot n°	Curing time									
	2	4	6	7	9	11	12	14	15	19
1 c			46.2		47.5		45.8			35.5
s					38.0		37.3			24.7
2 c	58.0	48.0		44.6		44.7		39.2		
s				37.3		34.3		30.2		
3 c			45.9		47.4		45.7		47.4	40.5
s					36.3		33.8		39.6	29.8
4 c	56.4	49.9		46.0		48.3		46.5		
s				33.8		37.1		30.7		
5 c			47.9		49.8		45.0		40.7	36.7
s					38.3		34.3		31.2	26.7
6 c	56.4	47.8		49.0		48.3		40.8		
s				39.9		36.3		31.4		
7 c			49.6		49.9		48.5		41.4	39.5
s					38.3		39.8		36.3	28.5
8 c	55.9	49.3		51.8		45.0		41.5		
s				36.7		34.7		31.2		



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TABLE 7  
LOT 1

Curing time	Weight losses	
	Per cent	
6	19.9	
10	25.9	
14	35.8	
20	41.8	
24	43.1	

TABLE 8  
LOT 2

Sub-lot	Weight losses									
	Curing time									
	4	6	7	9	11	12	14	15	19	
1		15.6				33.1		36.4	40.8	
2	17.9		24.6		31.7		35.1			
3		27.2		26.1		31.0		38.0	37.7	
4	16.9		22.6		34.4		33.7			
5		21.3		27.3		32.3		37.6	43.2	
6	21.4		29.3		39.2		38.5			
7		23.0		26.4		34.3		36.6	39.2	
8	23.7		27.3		36.7		37.5			

TABLE 9  
LOT 1

Curing time	pH value	
	c	s
2		6.00
6	5.60	5.76
10	4.80	4.90
14	4.80	4.80
20	4.75	4.80
24	4.75	4.80

TABLE 10  
LOT 2 pH values

Sub-lot n°	Curing time									
	2	4	6	7	9	11	12	14	15	19
1 c			4.85		4.78		4.80		4.70	4.77
s					4.85		4.85		4.75	4.88
2 c	5.66	4.90		4.85		4.80		4.72		
s				4.80		4.80		4.81		
3 c			4.80		4.80		4.66		4.65	4.67
s					4.90		4.75		4.70	4.77
4 c	5.90	5.89		4.65		4.60		4.67		
s				4.80		4.80		4.81		
5 c			4.80		4.80		4.80		4.70	4.73
s					4.95		4.90		4.80	4.75
6 c	5.95	4.90		4.70		4.75		4.78		
s				4.85		4.80		4.82		
7 c			4.80		4.82		4.70		4.80	4.70
s					4.97		4.80		4.90	4.77
8 c	5.90	4.85		4.75		4.75		4.75		
s				4.87		4.85		4.84		

TABLE 11  
LOT 1

Curing time	Na Cl content	
	c	s
0		2.03
2		2.90
6	3.45	3.59
10	3.71	4.26
14	4.20	4.78
20	4.40	4.92
24	4.60	5.51

S U M M A R Y

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CHANGES PRODUCED DURING CURING OF DRY SAUSAGES.

This paper deals with some factors related to the maturation of dry sausages, with specific reference to the Spanish sausage named "chorizo".

Firstly, some details concerning the "chorizo" manufacturing process, are analyzed.

Special emphasis is laid on the biological problems of maturation, and a description is given of the investigation of the genera which have been found.

Two types of germs deemed to be responsible for maturation have been isolated : one of these might belong in view of its characteristics, to the genus *Arthrobacter*, with a marked effect on the sugars, and the other appears to be similar to *Micrococcus halodemitrificans*. Both of these genera grow well in media containing 7 % sodium chloride.

Factors influencing the microbial development are also studied, equilibrium relative humidity, total humidity, pH values, and sodium chloride contents.

R E S U M E

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MODIFICATIONS QUI S'OPERENT PENDANT LE MURISSAGE DES SAUCISSES

Dans cet article on étudie quelques facteurs ayant trait à la maturation des saucisses secs, tout particulièrement du produit espagnole appelé "chorizo".

On analyse, tout d'abord, quelques détails concernant le processus de fabrication du "chorizo".

On met l'accent spécialement sur les problèmes biologiques regardant la maturation et on expose les résultats de l'investigation des genres retrouvés.

On a isolé deux types de germes que l'on considère comme responsables de la maturation : l'un peut appartenir, en vertu de ses caractéristiques, au genre *Arthrobacter*, ayant une action marquée sur les sucres; l'autre paraît être semblable au genre *Micrococcus halodemitrificans*. Les deux genres croissent assez bien dans des milieux contenant 7 % de chlorure de sodium.

On étudie aussi des facteurs influant sur le développement microbien : humidité relative d'équilibre, humidité totale, pH et teneur en chlorure de sodium.

ZUSAMMENFASSUNG  
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WAHREND DES REIFENS VON TROCKENER WURST AUGETRETENE VERÄNDERUNGEN

In der vorliegenden Arbeit werden einige Faktoren studiert, die mit der Reife von trockenen Wurstwaren zusammenhängen, wobei wir uns in diesem Fall speziell auf "chorizo" beziehen.

An erster Stelle werden einige Einzelheiten des Herstellungsprozesses des "chorizo" analysiert.

Besonderer Nachdruck wird auf die mikrobiologischen Probleme des Reife-  
prozesses gelegt, wobei die gefundenen Stoffe untersucht werden.

Es wurden zwei Keime besprochen, die wir fuer die Reife als ausschlaggebend betrachten; einer kann infolge seiner Charakteristiken zu der Familie der *Arthrobacter* gehörend erachtet werden mit einer bedeutenden Auswirkung auf den Zuckergehalt, während der andere dem *Micrococcus halodemitrificans* ähnlich zu sein scheint. Beide Keime wachsen gut in einer Umgebung, die 7 % Kochsalz enthält.

Faktoren, die mikrobische Entwicklung beeinflussen, werden ebenfalls untersucht, ebenso der Gleichgewichts-Feuchtigkeitsgrad, der Gesamt-Feuchtigkeitsgrad, der pH-Wert und der Kochsalzgehalt.



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